2020-06-08

Welcome to Week 12 of the COVID-19 Journey

How have I forgotten ballet?? Music and dance go together like a hand and glove. Here is the New York City Ballet in George Balanchine's 'Rubies' with music by Igor Stravinsky: https://www.youtube.com/watch?v=gw74CUCEqoM enjoy the aural and visual pleasure.

Here is a good STAT piece on what went wrong with the review process on the two retracted papers that used the Surgisphere database and one of the co-authors has his position terminated at University of Utah.

Now that things are reopening, people will be faced with making individual decisions about what constitutes personal pandemic risk. Here is a survey of 511 epidemiologists conducted by The New York Times that provides some sound information (kudos to an alert newsletter reader for seeing this before I did!!!).

As I run a small home investment office and serve on an advisory committee for a biomedical research endowment, I track a number of sources of financial information. Barry Ritholtz has a podcast at Bloomberg News and has, in recent weeks, had a number of high-profile investors and managers on that have discussed the COVID-19 pandemic. The two most recent ones with Jonathan Litt and David Rosenberg are especially worth listening to. We are going to see a fair number of disruptions in the next year. Litt believes that many companies have had success with remote work and that offices may never come back to normal occupancy. This has implications for a variety of economic activities within large metro areas.

There are only a short number of papers today but another positive tocilizumab study. I have one observation about ongoing trials. We have not seen a Data Safety Monitoring Board (DSMB) intervene to stop a trial for either remarkable efficacy or adverse safety. Results from one of the remdesivir trials was made public but they were rather marginal. I suspect this might mean we may not get any game changing results from the current therapies under investigation.

MODELING

- Anthropogenic pollution has frequently been linked to myriad human ailments despite clear mechanistic links are yet lacking, a fact that severely downgraded its actual relevance. Now a prominent unnoticed sub-weekly cycle (SWC) of 3.5 days is uncovered in the long-term epidemiological records of Kawasaki disease (KD) in Japan, a mysterious vasculitis of yet unknown origin. After ruling out the effect of reporting biases, the analysis of Light Detection and Ranging (LIDAR) atmospheric profiles further confirms that this variability is linked to atmospheric particles with an aerodynamic diameter less than 1 micron. SWC accounts for 20% of the variance in KD and its contribution is stable throughout the entire epidemiological record dating back to 1970, both at the prefecture level and for entire Japan. KD maxima in 2010-2016

always occur in full synchrony with LIDAR particle arrival in diverse locations such as Tokyo, Toyama and Tsukuba as well as for the entire of Japan. Rapid intrusion of aerosols from heights up to 6km to the surface is observed with KD admissions co-varying with their metal chemical composition. While regional intensity of winds has not changed in the interval 1979-2015, our study instead points for the first time to increased anthropogenic pollution as a necessary co-factor in the occurrence of KD and sets the field to associate other similar human vasculitis. [**note: I have no idea how this one was posted in a COVID-19 database but since Kawasaki disease has been associated with SARS-CoV-2 infection it is nevertheless an interesting finding. Maybe everything is linked to anthropogenic pollution! I found the paper interesting reading.**] https://www.medrxiv.org/content/10.1101/2020.06.04.20122325v1

- Objectives: To describe our experience with a coronavirus disease 2019 (COVID-19) outbreak within a large rheumatology department, early in the pandemic. Methods: Symptomatic and asymptomatic healthcare workers (HCWs) had a naso-oropharyngeal swab for detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and were followed clinically. Reverse transcription polymerase-chain reaction (RT-PCR) was repeated to document cure, and serological response was assessed. Patients with risk contacts within the department in the 14 days preceding the outbreak were screened for COVID-19 symptoms. Results: 14/34 HCWs (41%; 40±14 years, 71% female) tested positive for SARS-CoV-2, and 11/34 (32%) developed symptoms but were RT-PCR-negative. Half of RT-PCR-positive HCWs did not report fever, cough, or dyspnoea before testing, which were absent in 3/14 cases (21%). Mild disease prevailed (79%), but 3 HCWs had moderate disease requiring further assessment, which excluded severe complications. Nevertheless, symptom duration (28±18 days), viral shedding (31±10 days post-symptom onset, range 15-51) and work absence (29±28 days) were prolonged. 13/14 (93%) of RT-PCR-positive and none of the RT-PCR-negative HCWs had a positive humoral response, with higher IgG-index in individuals over 50 years (14.5±7.7 vs 5.0±4.4, p=0.012). Of 617 rheumatic patients, 8 (1.3%) developed COVID-19 symptoms (1/8 hospitalisation, 8/8 complete recovery), following a consultation/procedure with an asymptomatic (7/8) or mildly-symptomatic (1/8) HCW. Conclusions: A COVID-19 outbreak can occur among HCWs and rheumatic patients, swiftly spreading over the presymptomatic stage. Mild disease without typical symptoms should be recognised, and may evolve with delayed viral shedding, prolonged recovery, and adequate immune response in most individuals. [**note: a cautionary report of a COVID-19 outbreak in a large Lisbon rheumatology clinic. Large number of staff members were infected along with some patients. Some of the patients were on drug therapy but no mention was made about HCQ so we don't know if there was any patient level protection.**]
  https://www.medrxiv.org/content/10.1101/2020.06.05.20107011v1

NEWLY REGISTERED CLINICAL TRIALS

- Will check tomorrow.

CLINICAL TRIAL RESULTS

- Of 103 patients who were randomized (median age, 70 years; 60 [58.3%] male), 101 (98.1%) completed the trial. Clinical improvement occurred within 28 days in 51.9% (27/52) of the convalescent plasma group vs 43.1% (22/51) in the control group (difference, 8.8% [95% CI, −10.4% to 28.0%]; hazard ratio [HR], 1.40 [95% CI, 0.79-2.49]; *P* = .26). Among those with severe

disease, the primary outcome occurred in 91.3% (21/23) of the convalescent plasma group vs 68.2% (15/22) of the control group (HR, 2.15 [95% CI, 1.07-4.32]; *P* = .03); among those with life-threatening disease the primary outcome occurred in 20.7% (6/29) of the convalescent plasma group vs 24.1% (7/29) of the control group (HR, 0.88 [95% CI, 0.30-2.63]; *P* = .83) (*P* for interaction = .17). There was no significant difference in 28-day mortality (15.7% vs 24.0%; OR, 0.65 [95% CI, 0.29-1.46]; *P* = .30) or time from randomization to discharge (51.0% vs 36.0% discharged by day 28; HR, 1.61 [95% CI, 0.88-2.93]; *P* = .12). Convalescent plasma treatment was associated with a negative conversion rate of viral PCR at 72 hours in 87.2% of the convalescent plasma group vs 37.5% of the control group (OR, 11.39 [95% CI, 3.91-33.18]; *P* < .001). Two patients in the convalescent plasma group experienced adverse events within hours after transfusion that improved with supportive care. Among patients with severe or life-threatening COVID-19, convalescent plasma therapy added to standard treatment, compared with standard treatment alone, did not result in a statistically significant improvement in time to clinical improvement within 28 days. Interpretation is limited by early termination of the trial, which may have been underpowered to detect a clinically important difference. [**note: this is from China and contradicts findings from other groups. More confounding results to add to the kettle brew**] https://jamanetwork.com/journals/jama/article-abstract/2766943 but.... Read the editorial on this paper which expresses some optimism https://jamanetwork.com/journals/jama/fullarticle/2766940

- Tocilizumab is an IL-6 receptor antagonist with the ability to suppress the cytokine storm in critically ill patients infected with SARS-CoV-2. Methods: We evaluated patients treated with tocilizumab for a SARS-CoV-2 infection who were admitted between 3/13/20 and 4/16/20. This was a multi-center study with data collected by chart review both retrospectively and concurrently. Parameters evaluated included age, sex, race, use of mechanical ventilation (MV), usage of steroids and vasopressors, inflammatory markers, and comorbidities. Early dosing was defined as a tocilizumab dose administered prior to or within one (1) day of intubation. Late dosing was defined as a dose administered greater than one (1) day after intubation. In the absence of mechanical ventilation, the timing of the dose was related to the patient's date of admission only. Results: We evaluated 145 patients. The average age was 58.1 years, 64% were male, 68.3% had comorbidities, and 60% received steroid therapy. Disposition of patients was 48.3% discharged and 29.3% expired, of which 43.9% were African American. Mechanical ventilation was required in 55.9%, of which 34.5% expired. Avoidance of MV (p value = 0.002) and increased survival (p value < 0.001) was statistically associated with early dosing. Conclusions: Tocilizumab therapy was effective at decreasing mortality and should be instituted early in the management of critically ill COVID-19 patients. [**note: another positive paper on tocilizumab. I wonder if this trend will hold up and if the data are so good why hasn't a DSMB in one of the registered trials stopped and released interim results. At least this paper has a decent number of patients.**]
  https://www.medrxiv.org/content/10.1101/2020.06.05.20122622v1
- The continuous outbreak of COVID-19 poses a devastating threat to the global public health, and there's no special therapeutic drugs.This paper is to explore the effect of high fiber whey short peptide enteral nutrition on the prognosis of patients with COVID-19, to find ways to prevent patients from progressing to severe illness, and to reduce the harm of epidemic situation to human beings. Methods: The course of fighting against COVID-19 in our hospital for 49 days was

reviewed. Three nutritional interventions including five-step nutrition treatment, early intervention of whole protein, and high fiber whey short peptide intervention were conducted consecutively.The effect of high fiber whey short peptide on nutrition, immune function and prognosis of patients with COVID-19 was compared with that of whole protein intervention. Results: High fiber whey short peptide group when compared with whey protein group, can significantly improve prealbumin in patients with covid-19, improve the negative nitrogen balance of patients, and reduce the average period of turning negative by 39.06%. Conclusion: High fiber whey short peptide can significantly improve the recovery speed of patients with covid-19. [**note: data from Hubei suggests that a high fiber whey short peptide may have a positive impact on disease recovery.**]
https://www.medrxiv.org/content/10.1101/2020.06.03.20083980v1

- Individuals with advanced age and comorbidities face higher risk of death from COVID-19, especially once ventilator-dependent. Respiratory decline in COVID-19 is mediated by a pneumonic aberrant immune cytokine storm. Low-dose radiation was used to treat pneumonia in the pre-antibiotic era. Radiation immunomodulatory effects may improve outcomes in COVID-19. Methods: We performed a single-institution phase I/II trial evaluating the safety and efficacy of single-fraction, low-dose, whole-lung radiation for COVID-19 pneumonia. Eligible patients were hospitalized, had radiographic pneumonic infiltrates, required supplemental oxygen, and were clinically deteriorating. Results: Of nine patients screened, five were treated with whole-lung radiation from April 23-28, 2020 and followed for 7 days. Median age was 90 (range 64-94); four were nursing home residents with multiple comorbidities. Within 24 hours of radiation, three patients (60%) weaned from supplemental oxygen to ambient air, four (80%) exhibited radiographic improvement, and median Glasgow coma score improved from 10 to 14. A fourth patient (80% overall recovery) weaned from oxygen at hour 96. Mean time to clinical recovery was 35 hours. There were no acute skin, pulmonary, GI, GU toxicities. Conclusions: In a pilot trial of five oxygen-dependent patients with COVID-19 pneumonia, low-dose whole-lung radiation led to rapid improvement in clinical status, encephalopathy, and radiographic infiltrates without acute toxicity. Low-dose whole-lung radiation is safe, shows early promise of efficacy, and warrants further study in larger prospective trials. NCT04366791 [**note: I haven't covered this before. There are a scattering of clinical trials looking at low dose radiation. It's out of my area of expertise but looks like worth purusing.]**
https://www.medrxiv.org/content/10.1101/2020.06.03.20116988v1

DRUG DEVELOPMENT

- Here is another article on Human Challenge Trials for SARS-CoV-2 vaccine development.
https://jamanetwork.com/journals/jama/fullarticle/2767024
- Relevance: Management of symptoms like anxiety, delirium and agitation cannot be neglected in COVID-19 patients. Antipsychotics are usually used for the pharmacological management of delirium, and confusion and behavioral disturbances. The selection of concomitant COVID-19 medications and antipsychotics should be evidence-based and closely monitored Objective: To systematically review evidence-based available on drug-drug interactions between COVID-19 treatments and antipsychotics. Evidence Review: Three databases were consulted: (a) Lexicomp Drug Interactions, (b) Micromedex Solutions Drugs Interactions, (c) Liverpool Drug Interaction Group for COVID-19 therapies. To acquire more information on QT prolongation and TdP, the

CredibleMeds QTDrugs List was searched. Based on the information collected, the authors made a recommendation agreed to by consensus. In addition, a systematic review was conducted to find the clinical outcomes of drug-drug interactions between COVID-19 treatments and antipsychotics Results: The main interaction between COVID-19 drugs and antipsychotics are the risk of QT prolongation and TdP, and CYP interactions. Remdesivir, favipiravir, baricinitib, and anakinra can be used concomitantly with antipsychotics with no risk of drug-drug interaction (except for hematological risk with clozapine and baricinitib). Tocilizumab is rather safe to use in combination with antipsychotics, although it can restore the activity of CYP3A4 and therefore its substrate metabolism may increase. The most demanding COVID-19 treatments for co-administration with antipsychotics are chloroquine, hydroxychloroquine, azithromycin (all prolong QT interval) and lopinavir / ritonavir (CYP interaction and risk of QT prolongation). Conclusions: We urge to development of evidence-based guidelines that can help clinicians decide the safest treatment combination and monitoring necessary for each particular patient. The selection of concomitant COVID-19 medications and antipsychotics should be evidence-based and closely monitored. [**note: there has been a lot of spent time on HCQ safety with and without added antibiotics. This paper from Spain focuses on other drug interactions that might be of concern to clinicians. More needs to be done in this area in the absence of a true standard of practice.**] https://www.medrxiv.org/content/10.1101/2020.06.04.20122416v1

DIAGNOSTIC DEVELOPMENT

- Background The clinical performance of six molecular diagnostic tests and a rapid antigen test for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) were clinically evaluated for the diagnosis of coronavirus disease 2019 (COVID-19) in self-collected saliva. Methods Saliva samples from 103 patients with laboratory-confirmed COVID-19 (15 asymptomatic and 88 symptomatic) were collected on the day of hospital admission. SARS-CoV-2 RNA in saliva was detected using a quantitative reverse-transcription polymerase chain reaction (RT-qPCR) laboratory-developed test (LDT), a cobas SARS-CoV-2 high-throughput system, three direct RT-qPCR kits, and reverse-transcription loop mediated isothermal amplification (RT-LAMP). Viral antigen was detected by a rapid antigen immunochromatographic assay. Results Of the 103 samples, viral RNA was detected in 50.5-81.6% of the specimens by molecular diagnostic tests and an antigen was detected in 11.7% of the specimens by the rapid antigen test. Viral RNA was detected at a significantly higher percentage (65.6–93.4%) in specimens collected within 9 d of symptom onset compared to that of specimens collected after at least 10 d of symptom onset (22.2–66.7%) and that of asymptomatic patients (40.0–66.7%). Viral RNA was more frequently detected in saliva from males than females. Conclusions Self-collected saliva is an alternative specimen diagnosing COVID-19. LDT RT-qPCR, cobas SARS-CoV-2 high-throughput system, direct RT-qPCR except for one commercial kit, and RT-LAMP showed sufficient sensitivity in clinical use to be selectively used according to clinical settings and facilities. The rapid antigen test alone is not recommended for initial COVID-19 diagnosis because of its low sensitivity. [**note: some good data on the utility of testing saliva for SARS-CoV-2 via PCR and one antigen assay. The antigen assay performed poorly.**] https://www.medrxiv.org/content/10.1101/2020.06.06.20124123v1

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2020-06-09

Why should one move a Steinway concert grand to the middle of the woods? Silly question, it is to hear the wonderful French-Georgian pianist, Khatia Buniastishvili, play some wonderful solo pieces. I've been listening to a lot of her concerti over YouTube and she has both the technique and feeling for this instrument. Here is Das Waldkonzert: https://www.youtube.com/watch?v=eYlIk19CbBI Spectacular setting, wonderful playing and an appreciative audience.

It looks as if the protracted shutdown in the US did some good. Approximately 60 million cases of COVID-19 were avoided according to this study by a mostly University of California group. A separate study from the UK shows the impact on this approach in Europe. Here is a good commentary on the two papers from the Washington Post.

Can contact tracing work in Las Vegas? How long before business offices return to normal?

A pathological look at COVID-19. A JAMA interview with Tony Faucci on several topics and a JAMA editorial on inflammatory multisystem syndrome in children associated with SARS-CoV-2 infection (this also has links to two published papers in this week's journal on clinical information).

I always have a fondness for Tuesdays as there are usually a bunch of preprints loaded onto servers during the day on Monday. Lots of interesting reads!!!

MODELING

- The COVID-19 pandemic caused by the novel coronavirus SARS-CoV-2 has spread globally, with >52,000 cases in California as of May 4, 2020. Here we investigate the genomic epidemiology of SARS-CoV-2 in Northern California from late January to mid-March 2020, using samples from 36 patients spanning 9 counties and the Grand Princess cruise ship. Phylogenetic analyses revealed the cryptic introduction of at least 7 different SARS-CoV-2 lineages into California, including epidemic WA1 strains associated with Washington State, with lack of a predominant lineage and limited transmission between communities. Lineages associated with outbreak clusters in 2 counties were defined by a single base substitution in the viral genome. *These findings support contact tracing, social distancing, and travel restrictions to contain SARS-CoV-2 spread in California and other states*. [**note: good study from a multi-author group on the phylogenetic lineage of SARS-CoV-2 in California. I am continually amazed at the tools molecular epidemiologist have.]**
  https://science.sciencemag.org/content/early/2020/06/05/science.abb9263 and this paper from two Italian researchers is a careful analysis of all available mutation data through May - https://www.biorxiv.org/content/10.1101/2020.06.08.140152v1.full.pdf [**note: it is worth quoting the final two sentences of this paper, "Our structural analyses provide a rationale for such mutations, pointing to a weakening of the post-fusion assembly. However, only experiments on cellular systems will clarify whether this may be a virus strategy for reducing its membrane fusion capacity, thus lowering its virulence." Intriguing and it comports with a paper I posted several days ago that pointed to a similar effect. It's possible that there are**

now circulating versions of SARS-CoV-2 that are less virulent but still may trigger an immune response.] and here is more work on the same topic from Canadian researchers: https://www.biorxiv.org/content/10.1101/2020.06.08.140459v1

- Recent media articles have suggested that women-led countries are doing better in terms of their responses to the COVID-19 pandemic. We examine an ensemble of public health metrics to assess the control of COVID-19 epidemic in women- vs men-led countries worldwide based on data available up to June 3. The median of the distribution of median time-varying effective reproduction number for women and men led countries were 0.89 and 1.14 respectively with the 95% two-sample bootstrap-based confidence interval for the difference (women - men) being [-0.335, 0.028]. In terms of scale of testing, the median percentage of population tested were 3.28% (women), 1.59% (men) [95% CI: (-1.285%, 3.600%)] with test positive rates of 2.69% (women) and 4.94% (men) respectively. *It appears that though statistically not significant, countries led by women have an edge over countries led by men in terms of public health metrics for controlling the spread of the COVID-19 pandemic worldwide*. [**note: with a title '*Are women leaders significantly better at controlling the contagion,*' this is mandatory reading!!**] https://www.medrxiv.org/content/10.1101/2020.06.06.20124487v1
- After the outbreak of COVID-19 and the passing of a few months with this pandemic; the world has started to adopt strategies to live with the virus. The WHO has also accepted that the pandemic caused by the novel coronavirus is going to last longer, and suggested that one needs to learn to live with this virus. Accepting this bitter truth that this pandemic is going to be a new normal and people of all ages can be infected by the new coronavirus; however, older people and those with chronic diseases are more vulnerable to the virus. The study tries to access the household with at least one patient with few selected chronic diseases in India, which are presumed to be at a higher risk of losing at least one individual if this pandemic scenario is going to last long and spread is wider. The study used nationally representative data (NSSO) for information on morbidity and other health-related issues. Data from the official website of the Ministry of Health and Family Welfare dedicated to COVID-19 reports have been used to look into the recent happenings caused by COVID-19 pandemic in India. Bivariate analysis has been used to calculate household at risk, and binary logistic regression has been used for the likelihood of household at risk. The case-fatality ratio is calculated using the number of confirmed cases and the number deceased due to the same. *The study found that about 9.4% of Indian households are at a higher risk of losing at least one individual.* Older people (60+), males and households with better economic status are at a higher risk. The chronic condition varies by states and social-economic and demographic status. The share of households at higher risk was highest in Kerala (33.19%), followed by Andhra Pradesh (19.85%) and Chandigarh (19.05%). [**note: I have often written, 'we are all in this together.' It is often easy to forget the damage SARS-CoV-2 is doing to other countries that have fewer resources than the US. That an estimated 10% of Indian households may lose at least one individual is indeed sobering.**] https://www.medrxiv.org/content/10.1101/2020.06.08.20125203v1
- Outbreaks of emerging coronaviruses in the past two decades and the current pandemic of a novel coronavirus (SARS-CoV-2) that emerged in China highlight the importance of this viral family as a zoonotic public health threat. To gain a better understanding of coronavirus presence and diversity in wildlife at wildlife-human interfaces in three southern provinces in Viet Nam 2013-2014, we used consensus Polymerase Chain Reactions to detect coronavirus

sequences. In comparison to previous studies, we observed high proportions of positive samples among field rats (34.0%, 239/702) destined for human consumption and insectivorous bats in guano farms (74.8%, 234/313) adjacent to human dwellings. Most notably among field rats, the odds of coronavirus RNA detection significantly increased along the supply chain from field rats sold by traders (reference group; 20.7% positivity, 39/188) by a factor of 2.2 for field rats sold in large markets (32.0%, 116/363) and 10.0 for field rats sold and served in restaurants (55.6%, 84/151). Coronaviruses were detected in the majority of wildlife farms (60.7%, 17/28) and in the Malayan porcupines (6.0%, 20/331) and bamboo rats (6.3%, 6/96) that are farmed. We identified six known coronaviruses in bats and rodents, clustered in three *Coronaviridae* genera, including the *Alpha-*, *Beta-*, and *Gammacoronaviruses*. Our analysis also suggested either mixing of animal excreta in the environment or interspecies transmission of coronaviruses, as both bat and avian coronaviruses were detected in rodent feces in the trade. The mixing of multiple coronaviruses, and their apparent amplification along the wildlife supply chain into restaurants, suggests maximal risk for end consumers and likely underpins the mechanisms of zoonotic spillover to people. [**note: coronavirus testing of wildlife supply chains for human consumption in Vietnam. This is done on samples gathered in 2013-14 and is of interest in looking at possible animal reservoirs of this class of viruses. Menus in Vietnamese restaurants must be interesting. I will note that over the years I have eaten a number of hunted and fished animals including but not limited to frogs, pheasant, duck, squirrel and lots of different fish varieties. I had a grad school roommate who said all one needs is a good water proof tent, a dog, a fishing rod and a nice 12 gauge shotgun for wilderness survival. All I have left is the fishing rod.**] https://www.biorxiv.org/content/10.1101/2020.06.05.098590v1

NEWLY REGISTERED CLINICAL TRIALS

- This is a 14-day long prospective, multi-site, two-armed, randomized, open-label study that will enroll approximately 100 adult outpatients in Canada who have received a positive SARS-CoV-2 test result within the preceding 72 hours. Participants will be randomized (1:1) to receive either icosapent ethyl (4 g BID for 3 days, then 2 g BID for the subsequent 11 days) or usual care. Blood samples will be collected to determine if icosapent ethyl use lowers circulating pro-inflammatory biomarkers. [**note: this is a Canadian study and it is a fish-oil derived drug approved for the treatment of hypertriglyceridemia.**] NCT04412018
- This Phase II randomized, placebo-controlled, double-blind study will assess whether topical GLS-1200 applied via nasal spray atomizer is well-tolerated and can reduce the incidence of confirmed SARS-CoV-2 infection. Subjects will be randomized to either the GLS-1200 or placebo group in a 2:1 ratio with a target enrollment of 225 subjects. Subjects will self-administer study drug three times daily for 4 weeks. [**note: I don't know much more about this than what is at the link. This is a South Korean company that is sponsoring the trial.**] NCT04408183
- Systemic hyperinflammation is a hallmark of more severe stages of COVID-19 leading to acute respiratory distress syndrome, mechanical ventilation and ultimately death. In this stage, COVID-19 is associated with a decrease in suppressor and regulatory T cell counts and an extensive release of proinflammatory cytokines and biomarkers called a cytokine storm, which is thought to be the major driver of severe pneumonia caused by SARS-CoV-2. C1 esterase inhibitor (C1INH) is a member of the serpin superfamily of serine-protease inhibitors and is a strong inhibitor of the complement System (CS) and the kinin-kallikrein (KK) System. Conestat alfa is a

recombinant human C1INH, that shares an identical protein structure with plasma-derived C1INH. The rationale of the current trial is based upon the following assumptions: In the context of COVID-19, conestat alfa treatment may 1) dampen uncontrolled complement activation and collateral lung damage and 2) reduce capillary leakage and subsequent pulmonary edema by direct inhibition of KK system. The aim of this study is to analyze administration of conestat alfa for 72 hours in addition to standard of care in patients hospitalized with non-critical SARS-CoV-2 pneumonia (WHO Ordinal Scale Score 3 or 4) and its association with clinical severity on day 7 after inclusion and the risk of disease progression to Acute Lung Injury (ALI) and Acute Respiratory Distress Syndrome (ARDS). [**note: this is a Swiss trial and all I know about the drug is at the above link.**] NCT04414631

CLINICAL TRIAL RESULTS

- Background Concerns over the safety of non-steroidal anti-inflammatory drug (NSAID) use during SARS-CoV-2 infection have been raised. Objectives To study whether use of NSAIDs is associated with adverse outcomes and mortality during SARS-CoV-2 infection. Design Population based cohort study Setting Danish administrative and health registries. Participants Individuals tested positive for SARS-CoV-2 during Feb 27, 2020 to Apr 29, 2020. Treated individuals (defined as a filled NSAID prescription up to 30 days before the SARS-CoV-2 test) were matched to up to 4 non-treated individuals on propensity scores based on age, sex, relevant comorbidities and prescription fills. Outcome measures The main outcome was 30-day mortality and treated individuals were compared to untreated individuals using risk ratios (RR) and risk differences (RD). Secondary outcomes included hospitalisation, intensive care unit (ICU) admission, mechanical ventilation and acute renal replacement therapy. Results A total of 9236 SARS-CoV-2 PCR positive individuals were eligible for inclusion. Of these, 248 (2.7%) had filled a prescription for NSAIDs and 535 (5.8%) died within 30 days. In the matched analyses, treatment with NSAIDs was not associated with 30-day mortality (RR 1.02, 95% CI 0.57 to 1.82; RD 0.1%, -3.5% to 3.7%), increased risk of hospitalisation (RR 1.16, 0.87 to 1.53; RD 3.3%, -3.4% to 10%), ICU-admission (RR 1.04, 0.54 to 2.02; RD 0.2%, -3.0% to 3.4%), mechanical ventilation (RR 1.14, 0.56 to 2.30; RD 0.5%, -2.5% to 3.6%), or renal replacement therapy (RR 0.86, 0.24 to 3.09; RD -0.2%, -2.0% to 1.6%). Conclusion Use of NSAIDs was not associated with 30-day mortality, hospitalisation, ICU-admission, mechanical ventilation or renal replacement therapy in Danish individuals tested positive for SARS-CoV-2. [**note: good data from Denmark for those of you who need to take NSAIDS for various orthopedic conditions!**]
  https://www.medrxiv.org/content/10.1101/2020.06.08.20115683v1
- SARS-CoV-2 infection can cause severe disease for which currently no specific therapy is available. The use of hydroxychloroquine to prevent or treat SARS-CoV-2 infection is controversial and its mode of action poorly understood. We demonstrate that hydroxychloroquine inhibits trained immunity at the functional and epigenetic level and is accompanied by profound changes in the cellular lipidome as well as reduced expression of interferon-stimulated genes. Trained immunity comprises a functional adaptation induced by epigenetic reprogramming which facilitates the anti-viral innate immune response. Our findings therefore suggest that hydroxychloroquine may not have a beneficial effect on the anti-viral immune response to SARS-CoV-2. [**note: Oh no, say it ain't so Joe! Not only might HCQ be ineffective but it could also have an adverse impact on anti-viral immune response!!! I**

wonder if the Duke 15000-person healthcare study is fully enrolled. Are there dropouts in light of all the new information?]
https://www.medrxiv.org/content/10.1101/2020.06.08.20122143v1

- Background: SARS-CoV-2 infection can be detected indirectly by measuring the host immune response. Anti-viral antibody concentrations generally correlate with host protection and viral neutralization, but in rare cases, antibodies can promote disease progression. Elucidation of the kinetics and magnitude of the SARS-CoV-2 antibody response is essential to understand the pathogenesis of COVID-19 and identify potential therapeutic targets. Methods: Sera (n=533) from patients with RT-PCR confirmed COVID-19 (n=153) were tested using a high-throughput quantitative IgM and IgG assay that detects antibodies to the spike protein receptor binding domain and nucleocapsid protein. Individual and serial samples covered the time of initial diagnosis, during the disease course, and following recovery. We evaluated antibody kinetics and correlation between magnitude of the response and disease severity. Results: Patterns of SARS-CoV-2 antibody production varied considerably. Among 52 patients with 3 or more serial specimens, 44 (84.6%) and 42 (80.8%) had observed IgM and IgG seroconversion at a median of 8 and 10 days, respectively. Compared to those with milder disease, peak measurements were significantly higher for patients admitted to the intensive care unit for all time intervals between 6 and 20 days for IgM, and all intervals after 5 days for IgG. Conclusions: High sensitivity assays with a robust dynamic range provide a comprehensive picture of host antibody response to SARS-CoV-2. *IgM and IgG responses were significantly higher in patients with severe than mild disease. These differences may affect strategies for seroprevalence studies, therapeutics and vaccine development*. [**note: very interesting finding! Faster antibody mobilization may not necessarily be a predictor of a better clinical outcome. Clearly more needs to be done to understand this phenomenon.**]
https://www.medrxiv.org/content/10.1101/2020.06.03.20121525v1 and here is a link to a large UK study of seroconversion following SARS-CoV-2 infection.
https://www.medrxiv.org/content/10.1101/2020.06.07.20124636v1 [**note: Longitudinal analysis identifies 2-7% of individuals who don not seroconvert even weeks after infection. They are younger and less severely affected than seroconverters.**] and yet more, this time from Quebec https://www.biorxiv.org/content/10.1101/2020.06.08.140244v1 [**note: cross sectional study on 90 infected individuals. The vast majority of infected individuals elicited anti-Spike antibodies within 2 weeks after the onset of symptoms. The levels of receptor-binding domain (RBD)-specific IgG persisted overtime, while the levels of anti-RBD IgM decreased after symptoms resolution. Some of the elicited antibodies cross-reacted with other human coronaviruses in a genus-restrictive manner. While most of individuals developed neutralizing antibodies within the first two weeks of infection, the level of neutralizing activity was significantly decreased over time.]**
- Abstract Introduction In general SARS-CoV-2-infection during pregnancy is not considered to be an increased risk for severe maternal outcomes, but has been associated with an increased risk for fetal distress. So far, there is no direct evidence of intrauterine vertical transmission and the mechanisms leading to the adverse outcomes are not well understood Results An asymptomatic pregnant woman with preterm fetal distress during the COVID19 pandemic was included. We obtained multiple maternal, placental and neonatal swabs, which showed a median viral load in maternal blood, urine, oropharynx, fornix posterior over a period of 6 days was 5.0 log copies

/mL. The maternal side of the placenta had a viral load of 4.42 log copies /mL, while the fetal side had 7.15 log copies /mL. Maternal breast milk, feces and all neonatal samples tested negative. Serology of immunoglobulins against SARS-CoV-2 was tested positive in maternal blood, but negative in umbilical cord and neonatal blood. Pathological examination of the placenta included immunohistochemical investigation against SARS-CoV-2 antigen expression in combination with SARS-CoV-2 RNA in situ hybridization and transmission electron microscopy. It showed the presence of SARS-CoV-2 particles with generalized inflammation characterized by histiocytic intervillositis with diffuse perivillous fibrin depositions with damage to the syncytiotrophoblasts. Discussion Placental infection by SARS-CoV-2 lead to fibrin depositions hampering fetal-maternal gas exchange most likely resulted in fetal distress necessitating a premature emergency caesarean section. Postpartum, the neonate showed a clinical presentation resembling a pediatric inflammatory multisystem syndrome including coronary artery ectasia, most likely associated with SARS-CoV-2 (PIMS-TS) for which admittance and care on the Neonatal Intensive Care unit (NICU) was required, despite being negative for SARS-CoV-2. This highlights the need for awareness of adverse fetal and neonatal outcomes during the current COVID-19 pandemic, especially considering that the majority of pregnant women appear asymptomatic. [**note: it's one patient from The Netherlands but a cautionary story about maternal to fetal transmission.**]
https://www.medrxiv.org/content/10.1101/2020.06.08.20110437v1

- Background. Tocilizumab, a drug targeting interleukin-6 administrated in the right timeframe may be beneficial in coronavirus-disease-2019 (COVID-19). We aimed to assess its benefit, drawing from observations in compassionately treated patients. Methods: In a retrospective case-control study, treatment effect (tocilizumab 400mg, single-dose) was assessed using three statistical methods: propensity-score matching, Cox multivariable survival and inverse probability score weighting (IPSW) analyses. Were included all patients hospitalized with COVID-19, who presented severity criteria with SpO2<96% despite O2-support >6L/min for more than 6 hours. Were excluded patients in critical care medicine department and those under invasive mechanical ventilation. Primary outcome was a composite of mortality and ventilation, with a maximum follow-up of 28 days. Results: 246 patients were included (106 treated by tocilizumab). They were 67.6 +/-15.3 years-old, with 95 (38.5%) women. Delay between first symptoms and inclusion was 8.4 +/-4.5 days. Overall, 105 (42.7%) patients presented the primary outcome, with 71 (28.9%) deaths during the 28-days follow-up. Propensity-score-matched 84 pairs of comparable patients. In the matched cohort (n=168), tocilizumab was associated with fewer primary outcomes (hazard ratio (HR)=0.49 (95% confidence interval (95CI)=0.3-0.81), p-value=0.005). These results were similar in the overall cohort (n=246), with Cox multivariable analysis yielding a protective association between tocilizumab and primary outcome (adjusted HR=0.26 (95CI=0.135-0.51, p=0.0001), confirmed by IPSW analysis (p<0.0001). Analyses on mortality with 28-days follow-up yielded similar results. Conclusion: In this retrospective study, tocilizumab single-dose was associated with improved survival without mechanical ventilation in patients with severe COVID-19. [**note: another positive report on tocilizumab. One is led to wonder if this ought to be standard of care rather than remdesivir. I wish could learn to pronounce *tociizumab.***]
https://www.medrxiv.org/content/10.1101/2020.06.06.20122341v1

- Convalescing COVID-19 patients mount robust T cell responses against SARS-CoV-2, suggesting an important role for T cells in viral clearance. To date, the phenotypes of SARS-CoV-2-specific T cells remain poorly defined. Using 38-parameter CyTOF, we phenotyped longitudinal specimens of SARS-CoV-2-specific CD4+ and CD8+ T cells from four individuals who recovered from mild COVID-19. SARS-CoV-2-specific CD4+ T cells were exclusively Th1 cells, and predominantly Tcm with phenotypic features of robust helper function. SARS-CoV-2-specific CD8+ T cells were predominantly atypical Temra cells in a state of less terminal differentiation and therefore capable of expansion. Subsets of SARS-CoV-2-specific T cells exhibit features of being long-lived and capable of homeostatic proliferation consistent with their persistence for over two months. *Our results suggest that long-lived and robust T cell immunity is generated following natural SARS-CoV-2 infection, and support an important role for SARS-CoV-2-specific T cells in host control of COVID-19*. [**note: more information on the role of various parts of the immune system.**] https://www.biorxiv.org/content/10.1101/2020.06.08.138826v1
- COVID-19 is an ongoing global crisis in which the development of effective vaccines and therapeutics will depend critically on understanding the natural immunity to the virus, including the role of SARS-CoV-2-specific T cells. We have conducted a study of 42 patients following recovery from COVID-19, including 28 mild and 14 severe cases, comparing their T cell responses to those of 16 control donors. We assessed the immune memory of T cell responses using IFN gamma based assays with overlapping peptides spanning SARS-CoV-2 apart from ORF1. We found the breadth, magnitude and frequency of memory T cell responses from COVID-19 were significantly higher in severe compared to mild COVID-19 cases, and this effect was most marked in response to spike, membrane, and ORF3a proteins. Total and spike-specific T cell responses correlated with the anti-Spike, anti-Receptor Binding Domain (RBD) as well as anti-Nucleoprotein (NP) endpoint antibody titre ($p<0.001$, <0.001 and $=0.002$). We identified 39 separate peptides containing CD4+ and/or CD8+ epitopes, which strikingly included six immunodominant epitope clusters targeted by T cells in many donors, including 3 clusters in spike (recognised by 29%, 24%, 18% donors), two in the membrane protein (M, 32%, 47%) and one in the nucleoprotein (Np, 35%). CD8+ responses were further defined for their HLA restriction, including B*4001-restricted T cells showing central memory and effector memory phenotype. In mild cases, higher frequencies of multi-cytokine producing M- and NP-specific CD8+ T cells than spike-specific CD8+ T cells were observed. They furthermore showed a higher ratio of SARS-CoV-2-specific CD8+ to CD4+ T cell responses. Immunodominant epitope clusters and peptides containing T cell epitopes identified in this study will provide critical tools to study the role of virus-specific T cells in control and resolution of SARS-CoV-2 infections. The identification of T cell specificity and functionality associated with milder disease, *highlights the potential importance of including non-spike proteins within future COVID-19 vaccine design*. [**note: good paper from the UK that goes beyond the previous paper. The highlighted statement on possible inclusion of non-spike domains in vaccines is intriguing. This may argue that whole inactivated vaccine approaches might be a better approach but few of those are being developed.**] https://www.biorxiv.org/content/10.1101/2020.06.05.134551v1

DRUG DEVELOPMENT

- Like other betacoronaviruses, attachment and entry of SARS-CoV-2 is mediated by the spike glycoprotein (SGP). In addition to its well-documented interaction with its receptor, human

angiotensin converting enzyme 2 (hACE2), SGP has been found to bind to glycosaminoglycans like heparan sulfate, which is found on the surface of virtually all mammalian cells. Here, we pseudotyped SARS-CoV-2 SGP on a third generation lentiviral (pLV) vector and tested the impact of various sulfated polysaccharides on transduction efficiency in mammalian cells. The pLV vector pseudotyped SGP efficiently and produced high titers on HEK293T cells. Various sulfated polysaccharides potently neutralized pLV-S pseudotyped virus with clear structure-based differences in anti-viral activity and affinity to SGP. Concentration-response curves showed that pLV-S particles were efficiently neutralized by a range of concentrations of unfractionated heparin (UFH), enoxaparin, 6-O-desulfated UFH and 6-O-desulfated enoxaparin with an IC50 of 599 ng/L, 108 ug/L, 177 ng/L, and 586 ug/L respectively. The low serum bioavailability of intranasally administered UFH, along with data suggesting that the nasal epithelium is a portal for initial infection and transmission, suggest that intranasal administration of UFH may be an effective and safe prophylactic treatment. [**note: possible inhibitory mechanism for heparin**] https://www.biorxiv.org/content/10.1101/2020.06.08.140236v1

- The pandemic of SARS-CoV-2 coronavirus disease-2019 (COVID-19) caused by SARS-COV-2 continues to ravage many countries in the world. Mpro is an indispensable protein for viral translation in SARS-CoV-2 and a potential target in high-specificity anti-SARS-CoV-2 drug screening. In this study, to explore potential drugs for treating COVID-19, we elucidated the structure of SARS-CoV-2 Mpro and explored the interaction between Mpro and GC376, an antiviral drug used to treat a range of coronaviruses in Feline via inhibiting Mpro. The availability and safety of GC376 were proved by biochemical and cell experiments in vitro. We determined the structure of an important protein, Mpro, in SARS-CoV-2, and revealed the interaction of GC376 with the viral substrate and inhibition of the catalytic site of SARS-CoV-2 Mpro. [**note: GC376 is a bisulfite adduct of a peptidyl derivative, contain leucine and proline with an additional phenylalanine ring. I don't think there are any clinical trials of this compound.**] https://www.biorxiv.org/content/10.1101/2020.06.07.138677v1

DIAGNOSTIC DEVELOPMENT

- This study was conducted to validate an self-administrable saliva-based RT-qPCR test for the SARS-CoV2 virus under controlled laboratory conditions (analytical validation) according to federal guidance. An additional clinical study assessed positive (n=34) and negative (n=57) nasopharyngeal swab samples collected contemporaneously with saliva samples. Assessments for analytical specificity, sensitivity, cross reactivity and sample stability to simulate shipping conditions were conducted. Results: Positive and negative agreement with third-party laboratory results were reported as 97.1% and 96.5-98.2%, respectively. Limit of detection was established at 5 copies/µL. Stability through simulated shipping conditions found 100% concordance up to 56 hours after collection. Discussion: These data validate a self-collected saliva-based COVID-19 RT-qPCR assay that performs comparably well to an assay of health care-provider administered nasopharyngeal swab samples. Accordingly, the United States Food and Drug Administration granted emergency use authorization in June 2020. Use of the saliva-based assay overcomes barriers to the necessary widespread testing, including strained health care resources, supply chain disruptions of laboratory materials, testing and protective equipment and exposure risks due to close interpersonal contact. [**note: validation of a saliva collection**

method for RT-PCR testing by the company, Phosphorus Diagnostics.]
https://www.medrxiv.org/content/10.1101/2020.06.05.20122721v1

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2020-06-10

One thing that is great about YouTube are the interesting places that concerts are staged. We saw a woodsy setting yesterday. Today it's a pop up lunch room opera: https://www.youtube.com/watch?v=JNgCM7zp30M The kid's expressions are priceless!

Derek Lowe surveys what has been going on with monoclonal antibody development.

This story of teenager's reckless disregard for public health came across my newsfeed yesterday afternoon. I hope that cases are mild and that they have not put others at risk. Here is a report from Orange County CA (thank you Bill McBride of Calculated Risk for the HT) that highlights why the US will continue to suffer from the pandemic. I guess public health officials are not to be believed. What is it about wearing masks that is causing this type of reaction? Orange County continues to have significant cases of COVID-19 with associated burden on the ICU. Could this all be a result of the *Icarus Paradox* (**note: see https://www.medrxiv.org/content/10.1101/2020.06.08.20126029v1.full.pdf for how this may be related to SARS-CoV-2)***?* This news does not make me happy! ☹

Here is an interesting STAT article on the use of location data to look at COVID-19 response.

MODELING

- Background SARS-CoV-2 infection in Healthcare Workers (HCWs) is a public health concern during the pandemic. Little description has been made of their antibody response over time in the presence or absence detectable SARS-CoV-2 RNA and of symptoms. We followed a cohort of patient-facing HCWs at an acute hospital in London to measure seroconversion and RNA detection at the peak of the pandemic in London. Methods We enrolled 200 front-line HCWs between 26 March and 8 April 2020 and collected twice-weekly self-administered nose and throat swabs and monthly blood samples. Baseline and regular symptom data were also collected. Swabs were tested for SARS-CoV-2 RNA by polymerase chain reaction, and serum for IgM, IgA and IgG antibodies to the virus spike protein by enzyme-linked immunosorbent assay and flow cytometry. Findings We enrolled HCWs with a variety of roles who worked in areas where COVID-19 patients were admitted and cared for. During the first month of observation, 42/200 (21%) HCWs were PCR positive in at least one nose and throat swab. Only 8/42 HCW (19%) who were PCR positive during the study period had symptoms that met the current case definition. Of 181 HCWs who provided enrollment and follow-up blood samples, 82/181 (45.3%) were seropositive; 36/181 (19.9%) seroconverted during the study and 46/181 (25.4%) were seropositive at both time points. In 33 HCWs who had positive serology at baseline but were PCR negative, 32 remained PCR negative throughout follow-up. One HCW had a PCR positive

swab six days after enrollment, likely representing a waning infection. Interpretation The extremely high seropositivity and RNA detection in this cohort of front-line HCWs who worked during the peak of the pandemic brings policies to protect staff and patients in the hospital environment into acute focus. Our findings have implications for planning for the expected second wave and for future vaccination roll out campaigns in similar settings. The further evidence of asymptomatic SARS-CoV-2 infection indicates that asymptomatic surveillance of HCWs is essential while our study sets the foundations to answer pertinent questions around the duration of protective immune response and the risk of re-infection. [**note: here is another survey of healthcare workers, this time from the UK. It is fascinating that so many had positive serology tests but negative PCR. Perhaps this was infection prior to the start of the study.**] https://www.medrxiv.org/content/10.1101/2020.06.08.20120584v1 and here is another report on asymptomatic infection in nursing home worker in Colorado https://www.medrxiv.org/content/10.1101/2020.06.08.20125989v1 [**note: data suggests that asymptomatic individuals infected by SARS-CoV-2 may contribute to virus transmission within the workplace.**]

- As children are under-represented in current studies aiming to analyse transmission of SARS-coronavirus 2 (SARS-CoV-2), their contribution to transmission is unclear. Viral load, as measured by RT-PCR, can inform considerations regarding transmission, especially if existing knowledge of viral load in other respiratory diseases is taken into account. RT-PCR threshold cycle data from 3303 patients who tested positive for SARS-CoV-2 (out of 77,996 persons tested in total, drawn from across Germany) were analysed to examine the relationship between patient age and estimated viral load. Two PCR systems were used. In data from the PCR system predominantly used for community and cluster screening during the early phase of the epidemic (Roche LightCycler 480 II), when such screening was frequent practice, viral loads do not differ significantly in three comparisons between young and old age groups (differences in log10 viral loads between young and old estimated from raw viral load data and a Bayesian mixture model of gamma distributions collectively range between -0.11 and -0.43). Data from a second type of PCR system (Roche cobas 6800/8800), introduced into diagnostic testing on March 16, 2020 and used during the time when household and other contact testing was reduced, show a credible but small difference in the three comparisons between young and old age groups (differences, measured as above, collectively range between -0.43 and -0.83). This small difference may be due to differential patterns of PCR instrument utilization rather than to an actual difference in viral load. Considering household transmission data on influenza, which has a similar viral load kinetic to SARS-CoV-2, the viral load differences between age groups observed in this study are likely to be of limited relevance. Combined data from both PCR instruments show that viral loads of at least 250,000 copies, a threshold we previously established for the isolation of infectious virus in cell culture at more than 5% probability, were present across the study period in 29.0% of kindergarten-aged patients 0-6 years old (n=38), 37.3% of those aged 0-19 (n=150), and in 51.4% of those aged 20 and above (n=3153). The differences in these fractions may also be due to differences in test utilization. We conclude that a considerable percentage of infected people in all age groups, including those who are pre- or mild-symptomatic, carry viral loads likely to represent infectivity. *Based on these results and uncertainty about the remaining incidence, we recommend caution and careful monitoring during gradual lifting of non-pharmaceutical interventions. In particular, there is little evidence from the present study to*

support suggestions that children may not be as infectious as adults. [**note: decisions are being made to reopen schools and this is a cautionary paper that looks at viral load between young and old cohorts. It poses a difficult issue for me as our daughter is a special education teacher and pre-pandemic comes over for a weekly dinner. It may be when school reopens that we have to put a halt to this.**] https://www.medrxiv.org/content/10.1101/2020.06.08.20125484v1

NEWLY REGISTERED CLINICAL TRIALS

- Will check tomorrow.

CLINICAL TRIAL RESULTS

- Patients with severe COVID-19 have a hyperinflammatory immune response suggestive of macrophage activation. Bruton tyrosine kinase (BTK) regulates macrophage signaling and activation. Acalabrutinib, a selective BTK inhibitor, was administered off-label to 19 patients hospitalized with severe COVID-19 (11 on supplemental oxygen; 8 on mechanical ventilation), 18 of whom had increasing oxygen requirements at baseline. Over a 10-14 day treatment course, acalabrutinib improved oxygenation in a majority of patients, often within 1-3 days, and had no discernable toxicity. Measures of inflammation – C-reactive protein and IL-6 – normalized quickly in most patients, as did lymphopenia, in correlation with improved oxygenation. At the end of acalabrutinib treatment, 8/11 (72.7%) patients in the supplemental oxygen cohort had been discharged on room air, and 4/8 (50%) patients in the mechanical ventilation cohort had been successfully extubated, with 2/8 (25%) discharged on room air. Ex vivo analysis revealed significantly elevated BTK activity, as evidenced by autophosphorylation, and increased IL-6 production in blood monocytes from patients with severe COVID-19 compared with blood monocytes from healthy volunteers. These results suggest that targeting excessive host inflammation with a BTK inhibitor is a therapeutic strategy in severe COVID-19 and has led to a confirmatory international prospective randomized controlled clinical trial. [**note: a small sampling but to me increasing evidence that immune system blockers and modulators are the way to go for treatment.**] https://immunology.sciencemag.org/content/5/48/eabd0110
- Evaluate the risk factors of prolonged SARS-CoV-2 virus shedding and the impact of arbidol treatment on SARS-CoV-2 virus shedding. Methods: Data were retrospective collected from adults hospitalized with COVID-19 in Wuhan Union Hospital. We described the clinical features and SARS-CoV-2 RNA shedding of patients with COVID-19 and evaluated factors associated with prolonged virus shedding by multivariate regression analysis. Results: Among 238 patients, the median age was 55.5 years, 57.1% were female, 92.9% (221/238) used arbidol, 58.4% (139/238) used arbidol combination with interferon. The median time from illness onset to start arbidol was 8 days (IQR, 5-14 days) and the median duration of SARS-CoV-2 virus shedding was 23 days (IQR, 17.8-30 days). SARS-CoV-2 RNA clearance was significantly delayed in patients who received arbidol >7 days after illness onset, compared with those in whom arbidol treatment was started less than or equal to7 days after illness onset (HR, 1.738 [95% CI, 1.339-2.257], P < .001). Multivariate regression analysis revealed that prolonged viral shedding was significantly associated with initiation arbidol more than seven days after symptom onset (OR 2.078, 95% CI [1.114-3.876], P .004), more than 7 days from onset of symptoms to first medical visitation (OR 3.321, 95% CI[1.559-7.073], P .002), illness onset before Jan.31, 2020 (OR 3.223, 95% CI[1.450-7.163], P .021). Arbidol combination with interferon was also significantly associated with

shorter virus shedding (OR .402, 95% CI[.206-.787], P .008). Conclusions: Early initiation of arbidol and arbidol combination with interferon as well as consulting doctor timely after illness onset were helpful for SARS-CoV-2 clearance. [**note: Arbidol is the brand name for umifenovir, a Russian developed antiviral against influenza. There are a few clinical trials of the compound but since some of them are in China, it's unclear whether they are fully enrolled. This is the first paper that I can recall that points to its usefulness.**]
https://www.medrxiv.org/content/10.1101/2020.06.09.20076646v1

- Most patients with COVID-19, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), display neurological symptoms, and respiratory failure in certain cases could be of extra-pulmonary origin. With reports detecting SARS-CoV-2 in some post-mortem patient brains, the routes, targets and consequences of brain infection merit investigation. Hypothalamic neural circuits play key roles in sex differences, diabetes, hypertension, obesity and aging, all risk factors for severe COVID-19, besides being connected to brainstem cardiorespiratory centers. Here, human brain gene-expression analyses reveal that the hypothalamus and associated regions express angiotensin-converting enzyme 2 and transmembrane proteinase, serine 2, which mediate SARS-CoV-2 cellular entry, in correlation with several genes or pathways involved in physiological functions or viral pathogenesis. Immunolabeling in human and animal brains suggests that the hypothalamus could be central to SARS-CoV-2 brain invasion through multiple routes, and that sex hormones and metabolic diseases influence brain susceptibility. [**note: from France, a suggestion that the hypothalamus is the hub for SARS-CoV-2 brain infection.**]
https://www.biorxiv.org/content/10.1101/2020.06.08.139329v1
- A growing body of evidence indicates sex differences in the clinical outcomes of coronavirus disease 2019 (COVID-19)1-4. However, whether immune responses against SARS-CoV-2 differ between sexes, and whether such differences explain male susceptibility to COVID-19, is currently unknown. In this study, we examined sex differences in viral loads, SARS-CoV-2-specific antibody titers, plasma cytokines, as well as blood cell phenotyping in COVID-19 patients. By focusing our analysis on patients with mild to moderate disease who had not received immunomodulatory medications, our results revealed that male patients had higher plasma levels of innate immune cytokines and chemokines including IL-8, IL-18, and CCL5, along with more robust induction of non-classical monocytes. In contrast, female patients mounted significantly more robust T cell activation than male patients during SARS-CoV-2 infection, which was sustained in old age. Importantly, we found that a poor T cell response negatively correlated with patients age and was predictive of worse disease outcome in male patients, but not in female patients. Conversely, higher innate immune cytokines in female patients associated with worse disease progression, but not in male patients. These findings reveal a possible explanation underlying observed sex biases in COVID-19, and provide important basis for the development of sex-based approach to the treatment and care of men and women with COVID-19. [**note: we are getting closer to figuring out the immune and sex differential response to SARS-CoV-2 infection. This is an intriguing study.**]
https://www.medrxiv.org/content/10.1101/2020.06.06.20123414v1
- By comparison to pauci-symptomatic virus clearance by most individuals, Covid-19 has been proposed to reflect insufficient and/or pathologically exaggerated immune responses. Here we identify a consensus peripheral blood immune signature across 63 hospital-treated Covid-19 patients who were otherwise highly heterogeneous. The core signature conspicuously blended

adaptive B cell responses typical of virus infection or vaccination with discrete traits hitherto associated with sepsis, including monocyte and dendritic cell dampening, and hyperactivation and depletion of discrete T cell subsets. This blending of immuno-protective and immuno-pathogenic potentials was exemplified by near-universal CXCL10/IP10 upregulation, as occurred in SARS1 and MERS. Moreover, specific parameters including CXCL10/IP10 over-expression, T cell proliferation, and basophil and plasmacytoid dendritic cell depletion correlated, often prognostically, with Covid-19 progression, collectively composing a resource to inform SARS-CoV-2 pathobiology and risk-based patient stratification. [**note: from the UK, another data set on the immune system response and disease progression.**] https://www.medrxiv.org/content/10.1101/2020.06.08.20125112v1

- Background The management of hypoxic respiratory failure due to COVID-19 is not currently subject to consensus. International and national guidance has favoured early intubation, with concerns persisting over the use of CPAP. However, considering available evidence and local circumstances, early ward based CPAP and self proning was adopted in our institution. We aimed to evaluate the safety and efficacy of this approach. Methods In this retrospective observational study we included all patients admitted with a positive COVID-19 PCR. Negative patients were also included where clinical suspicion remained high. A large number of simple CPAP machines were used with entrained oxygen. Ward staff were supported in their use by physiotherapists and an intensive critical care outreach program. CPAP was initiated early via protocol, with the aim of preventing rather than responding to deterioration. Data was analysed descriptively. Results 559 patients admitted prior to 1/May/20 were included. 29.5% received CPAP, 7.2% were admitted to ICU and 4.8% were ventilated. Hospital mortality was 33.3%, ICU mortality 54.5%. Following CPAP, 64% of patients with moderate or severe ARDS at presentation, who were candidates for escalation, avoided intubation during their stay. Conclusion Figures for ICU admission, intubation and overall hospital mortality are significantly lower than those reported in a large and relevant comparator database, whilst ICU mortality is similar. This is despite our population having high levels of co-morbidity and ethnicities associated with poor outcomes. We advocate this approach as both effective and safe. [**note: good out of the box thinking to see what other clinical approaches are useful. Yes, it's a single center but clinicians should look to this as a viable approach to reducing the rust to intubation.**] https://www.medrxiv.org/content/10.1101/2020.06.05.20123307v1
- Several clinical studies have provided evidence for the antiviral effects of type I interferons (IFNs) in patients with respiratory coronaviruses. This study assessed the therapeutic efficacy of IFN-alpha 2b in patients infected with SARS-CoV-2 during the first month after the outbreak began in Cuba. Method This multicenter prospective observational study was conducted in 16 hospitals in 8 Cuban provinces. Participants were patients with confirmed SARS-CoV-2 infection detected from throat swab specimens by real time RT-PCR who gave informed consent and had no contraindications for IFN treatment. Patients received therapy as per the Cuban COVID protocol, that included a combination of oral antivirals (lopinavir/ritonavir and chloroquine) with intramuscular administration of IFN-alpha 2b (Heberon Alpha R, Center for Genetic Engineering and Biotechnology, Havana), 3 times per week, for 2 weeks. The primary endpoint was the proportion of patients discharged from hospital (without clinical and radiological symptoms and non-detectable virus by RT-PCR). The secondary endpoint was the case fatality rate (CFR), defined as the number of confirmed deaths divided by the number of confirmed

cases. Results From March 11th to April 14th, 814 patients were confirmed SARS-CoV-2 positive in Cuba, 761 (93.4%) were treated with Heberon Alpha R and 53 received the approved protocol without IFN treatment. The proportion of fully recovered patients was higher in the IFN-treated compared with non-IFN treated group (95.4% vs 26.1%, p<0.01). The CFR for all patients was 2.95%, and for those patients who received IFN-alpha 2b the CFR was reduce to 0.92. The estimated global CFR is 6.34% and 4.05% for the Americas reported by WHO and PAHO, respectively. In this study, 82 patients (10.1%) required intensive care and, of these, 42 (5.5%) were treated with IFN. Conclusions This report provides preliminary evidence for the therapeutic effectiveness of IFN alpha-2b for COVID-19 and suggests that the use of Heberon Alpha R may contribute to complete recovery. [**note: this looks like a robust trial from Cuba with 761 patients treated.**
https://www.medrxiv.org/content/10.1101/2020.05.29.20109199v1

- Ivermectin has been shown to inhibit the replication of SARS-CoV-2 in vitro but clinical response has not been previously evaluated. Objective: To determine whether Ivermectin is associated with lower mortality rate in patients hospitalized with COVID-19. Design and Setting: Retrospective cohort study of consecutive patients hospitalized at four Broward Health hospitals in South Florida with confirmed SARS-CoV-2. Enrollment dates were March 15, 2020 through May 11, 2020. Follow up data for all outcomes was May 19, 2020. Participants: 280 patients with confirmed SARS-CoV-2 infection (mean age 59.6 years [standard deviation 17.9], 45.4% female), of whom 173 were treated with ivermectin and 107 were usual care were reviewed. 27 identified patients were not reviewed due to multiple admissions, lack of confirmed COVID results during hospitalization, age less than 18, pregnancy, or incarceration. Exposure: Patients were categorized into two treatment groups based on whether they received at least one dose of ivermectin at any time during the hospitalization. Treatment decisions were at the discretion of the treating physicians. Severe pulmonary involvement at study entry was characterized as need for either FiO2 ≥50%, or noninvasive or invasive mechanical ventilation. Main Outcomes and Measures: The primary outcome was all-cause in-hospital mortality. Secondary outcomes included subgroup mortality in patients with severe pulmonary involvement and extubation rates for patients requiring invasive ventilation. Results: Univariate analysis showed lower mortality in the ivermectin group (25.2% versus 15.0%, OR 0.52, 95% CI 0.29-0.96, P=.03). Mortality was also lower among 75 patients with severe pulmonary disease treated with ivermectin (38.8% vs 80.7%, OR 0.15, CI 0.05-0.47, P=.001), but there was no significant difference in successful extubation rates (36.1% vs 15.4%, OR 3.11 (0.88-11.00), p=.07). After adjustment for between-group differences and mortality risks, the mortality difference remained significant for the entire cohort (OR 0.27, CI 0.09-0.85, p=.03; HR 0.37, CI 0.19-0.71, p=.03). Conclusions and Relevance: Ivermectin was associated with lower mortality during treatment of COVID-19, especially in patients who required higher inspired oxygen or ventilatory support. These findings require randomized controlled trials for confirmation. [**note: this is an open label study of ivermectin and not a registered trial. I think this is first clinical results paper that I've seen. It's hard to figure it out as there have been previous papers that based on the *in vitro* testing the pharmacological dose of ivermectin needs to be extraordinarily high. Most of the patients were also treated with HCQ/azithromycin as that was the standard of care at the time. There are controlled trials going on with ivermectin at a number of different trial sites.**] https://www.medrxiv.org/content/10.1101/2020.06.06.20124461v1

- To evaluate the effect of proton pump inhibitors on the course of common COVID-19. Methods: Clinical data of common COVID-19 patients admitted to the Shanghai public health clinical center for treatment from January 20, 2020 to March 16, 2020 were collected. A retrospective study was conducted and the patients were divided into two groups according to whether they used proton pump inhibitors or not. The differences in SARS-CoV-2 clearance and hospital stay between the two groups were compared by univariate and multivariate analyses. Results: A total of 154 COVID-19 common cases were included in this study, including 80 males (51.9%), 35 patients (22.7%) in the proton pump inhibitors group, and 119 patients (77.3%) in the control group. In the proton pump inhibitors group and the control group, the duration of SARS-CoV-2 clearance were 7(6-9) and 7(6-11) days, and the duration of hospital stay was 21(16-25) and 20(15-26) days, respectively. There was no significant difference between the two groups in the cumulative incidence of SARS-CoV-2 clearance and the cumulative incidence of discharge, and the same after Propensity Score Match, all $P > 0.05$. Multivariate analysis suggested that chronic gastropathy prolonged the duration of SARS-CoV-2 clearance, the HR was 20.924(3.547-123.447). Hypertension, chronic obstructive pulmonary disease, chronic liver disease and malignant tumor all increased the duration of hospital stay for COVID-19, and the HR were 1.820 (1.073-3.085), 4.370 (1.205-15.844), 9.011 (2.681-30.290) and 5.270 (1.237-22.456), respectively; the duration of hospital stay in COVID-19 patients was shortened by SARS-CoV-2 clearance, and the HR was 0.907 (0.869-0.947); all $P < 0.05$. Conclusion: Proton pump inhibitors use have no effect on the prolonging or shortening of the course of adults hospitalized with COVID-19. [**note: small cohort study from China showing that proton pump inhibitors don't have any effect on COVID-19 infection.**]
  https://www.medrxiv.org/content/10.1101/2020.06.07.20124776v1
- While there are no treatments with proven efficacy for patients with severe coronavirus disease 2019 (COVID 19), tocilizumab has been proposed as a candidate therapy, especially among patients with higher systemic inflammation. Methods We conducted a cohort study of patients hospitalized with COVID 19 in Spain. The primary outcome was time to death and the secondary outcome time to intensive care unit admission (ICU) or death. We used inverse probability weighting to fit marginal structural models adjusted for time varying covariates to determine the causal relationship between tocilizumab use and the outcomes. Results A total of 1,229 and 10,673 person/days were analyzed. In the adjusted marginal structural models, a significant interaction between tocilizumab use and high C reactive protein (CRP) levels was detected. Tocilizumab was associated with decreased risk of death (aHR 0.34, 95% CI 0.16 to 0.72, p=0.005) and ICU admission or death (aHR 0.38, 95% CI 0.19 to 0.81, p=0.011) among patients with baseline CRP >150 mg/L, but not among those with CRP ≤150 mg/L. Exploratory subgroup analyses yielded point estimates that were consistent with these findings. Conclusions In this large observational study, tocilizumab was associated with a lower risk of death or ICU or death in patients with higher CRP levels. While the results of ongoing clinical trials of tocilizumab in patients with COVID 19 will be important to establish its safety and efficacy, our findings have implications for the design of future clinical trials and support the use of tocilizumab among subjects with higher CRP levels. [**note: yet more data on the usefulness of tocilizumab.**]
  https://www.medrxiv.org/content/10.1101/2020.06.08.20125245v1

DRUG DEVELOPMENT

- Coronaviruses that infect humans belong to the Alpha-coronavirus (including HCoV-229E) and Beta-coronavirus (including SARS-CoV and SARS-CoV-2) genera. In particular, SARS-CoV-2 is currently a major threat to public health worldwide. However, no commercial vaccines against the coronaviruses that can infect humans are available. The spike (S) homotrimers bind to their receptors through the receptor-binding domain (RBD), which is believed to be a major target to block viral entry. In this study, we selected Alpha-coronavirus (HCoV-229E) and Beta-coronavirus (SARS-CoV and SARS-CoV-2) as models. Their RBDs were observed to adopt two different conformational states (lying or standing). Then, structural and immunological analyses were used to explore differences in the immune response with RBDs among these coronaviruses. Our results showed that more RBD-specific antibodies were induced by the S trimer with the RBD in the standing state (SARS-CoV and SARS-CoV-2) than the S trimer with the RBD in the lying state (HCoV-229E), and the affinity between the RBD-specific antibodies and S trimer was also higher in the SARS-CoV and SARS-CoV-2. In addition, we found that the ability of the HCoV-229E RBD to induce neutralizing antibodies was much lower and the intact and stable S1 subunit was essential for producing efficient neutralizing antibodies against HCoV-229E. Importantly, our results reveal different vaccine strategies for coronaviruses, and S-trimer is better than RBD as a target for vaccine development in Alpha-coronavirus. Our findings will provide important implications for future development of coronavirus vaccines. [**note: I continue to believe that the a lot of different approaches to vaccine development are needed for SARS-CoV-2. I worry that scientists are 'falling in love' with novel RNA and DNA vaccines which may not be optimal.**] https://www.biorxiv.org/content/10.1101/2020.06.09.141580v1
- Cholesterol 25-hydroxylase (CH25H) is an interferon-stimulated gene (ISG) that shows broad antiviral activities against a wide range of enveloped viruses. Here, using an ISG screen against VSV-SARS-CoV and VSV-SARS-CoV-2 chimeric viruses, we identified CH25H and its enzymatic product 25-hydroxycholesterol (25HC) as potent inhibitors of virus replication. Mechanistically, internalized 25HC accumulates in the late endosomes and blocks cholesterol export, thereby restricting SARS-CoV-2 spike protein catalyzed membrane fusion. Our results highlight a unique antiviral mechanism of 25HC and provide the molecular basis for its possible therapeutic development. [**note: another therapeutic approach from these St. Louis researchers.**] https://www.biorxiv.org/content/10.1101/2020.06.08.141077v1
- Molecular-level understanding of human neutralizing antibody responses to SARS-CoV-2 could accelerate vaccine design and facilitate drug discovery. We analyzed 294 SARS-CoV-2 antibodies and found that IGHV3-53 is the most frequently used IGHV gene for targeting the receptor binding domain (RBD) of the spike (S) protein. We determined crystal structures of two IGHV3-53 neutralizing antibodies +/- Fab CR3022 ranging from 2.33- to 3.11-angstrom resolution. The germline-encoded residues of IGHV3-53 dominate binding to the ACE2 binding site epitope with no overlap with the CR3022 epitope. Moreover, IGHV3-53 is used in combination with a very short CDR H3 and different light chains. Overall, IGHV3-53 represents a versatile public VH in neutralizing SARS-CoV-2 antibodies, where their specific germline features and minimal affinity maturation provide important insights for vaccine design and assessing outcomes. [**note: good work from Scripps with some antibody analysis may result in better vaccine design.**] https://www.biorxiv.org/content/10.1101/2020.06.08.141267v1
- The emergence of SARS-CoV-2 and the ensuing explosive epidemic of COVID19 disease has generated a need for assays to rapidly and conveniently measure the antiviral activity of SARS-

CoV-2-specific antibodies. Here, we describe a collection of approaches based on SARS-CoV-2 spike-pseudotyped, single-cycle, replication-defective human immunodeficiency virus type-1 (HIV-1) and vesicular stomatitis virus (VSV), as well as a replication-competent VSV/SARS-CoV-2 chimeric virus. While each surrogate virus exhibited subtle differences in the sensitivity with which neutralizing activity was detected, the neutralizing activity of both convalescent plasma and human monoclonal antibodies measured using each virus correlated quantitatively with neutralizing activity measured using an authentic SARS-CoV-2 neutralization assay. The assays described herein are adaptable to high throughput and are useful tools in the evaluation of serologic immunity conferred by vaccination or prior SARS-CoV-2 infection, as well as the potency of convalescent plasma or human monoclonal antibodies.[**note: another useful tool to evaluate neutralizing antibodies.]**
https://www.biorxiv.org/content/10.1101/2020.06.08.140871v1

DIAGNOSTIC DEVELOPMENT

- Nothing new here.

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2020-06-11

One of my loyal readers wants to hear and see the wonderful Chinese pianist Yuja Wang. Ms. Wang got her early training in China before moving to the US and studying at the Curtis Institute in Philiadelphia. As I am always happy to accept recommendations from my readers, here is Yuja Wang performing Rachmaninoff 3rd piano concerto with fingers flying over the keyboard. She is accompanied by the Vienna Philharmonic under the direction of Andrés Orozco-Estrada (it also has the Dvorak 'New World Symphony' as a bonus): https://www.youtube.com/watch?v=JKdpEvzKoj4

Will the drop in community SARS-CoV-2 infections cause problems for vaccine researchers? This Washington Post story points to this being the case.

Israel was looked to as a model of doing everything right to contain SARS-CoV-2. They moved on to broadly reopen the country and now are seeing jumps in viral infections and have closed some schools. We need to be ever-vigilant and maintain good public health practices. Here is a Washington Post story on what college openings in the US might look like.

A young woman whose lungs were irreparably damaged by COVID-19 received a double lung transplant at Northwestern.

Good news on the antibody front, Regeneron are beginning the first clinical trials of an anti-viral antibody cocktail! Here is a nice overview on what immunity to SARS-CoV-2 may look like courtesy of STAT. I need to read up on nanobodies, single chain antibody fragments, produced by llamas. There are more papers today and I'm not sure whether this approach has ever been tried clinically. Most of the work in the area relates to humanized mAbs produced in cell culture.

Mea Culpa Department: I was lax in not pointing out some of the problems with the Cuban interferon study that I posted yesterday. Another loyal reader noted that the trial was not very robust. Patients

were not given just interferon but also HCQ and lopinavir/ritonavir which further confounds the analysis. More importantly, "*Patients who did not receive interferon were older (median 66.9 years vs 42.9 years) with a higher incidence of comorbidities (56.6% vs 3.2%), such as high blood pressure, ischemic heart disease and diabetes mellitus."* By any measure this is not a *robust* study other than the number of patients being treated. It shows me that if something really looks good, it is time to take a dive into the data!! There are some registered controlled trials of several interferons going on and TIWWDCT!

MODELING

- Mass transportation is one of the areas that are badly hit by respiratory infectious disease outbreaks due to moderate to high exposure risk to pathogens brought about by the interaction among commuters. Here, we formulate agent-based models that simulate the spread of a respiratory infectious disease in a train wagon in the Manila Light Rail Transit System, and in a 49-seater public utility bus. We consider preventive measures such as implementation of social distancing, and limitation of interaction or movement among the commuters to investigate how these measures will inhibit disease transmission. We also consider the effect of protective gears and practices, crowd density, and prevalence of disease in the community on the possible number of newly-infected individuals. Our simulations show that (i) individuals must have protection with more than 90% effectiveness to inhibit transmission of the disease; (ii) social or physical distancing by more than 1m distance reduces the risk of being infected; (iii) minimizing movement or interaction with other passengers reduces the risk of transmission by 50%; (iv) passenger capacity should be less than 10-50% of the maximum seating capacity to reduce the number of infections depending on the level of imposed social distancing and passenger interaction; (v) vehicles with greater number of occupied seating capacity generate higher number of infections but vehicles with smaller dimensions have faster disease transmissions; and (vi) ideal set-up for a 24-seater train wagon (49-seater bus) is to allow a maximum of 12 (24) passengers, with little to no interaction among passengers, with social distancing of more than 1m distance apart, and each person has a protection with 90% effectiveness as much as possible. These results can aid policy makers in determining optimal strategies to minimize infections while maintaining transportation services during pandemics or disease outbreaks. [**note: I include this model as many people depend on mass transportation to travel to work. Not everyone has the luxury of working from home. Policy makers need to consider how to make mass transit safer during the pandemic era.**]
  https://www.medrxiv.org/content/10.1101/2020.06.09.20126334v1
- Further, heterogeneity in disease outcomes is influenced by race, though the relative contributions of structural/social and genetic factors remain unclear. Very recent unpublished work has identified two genetic risk loci that confer greater risk for respiratory failure in COVID-19: the ABO locus and the 3p21.31 locus. To understand how these loci might confer risk and whether this differs by race, we utilized proteomic profiling and genetic information from three cohorts including black and white participants to identify proteins influenced by these loci. We observed that variants in the ABO locus are associated with levels of CD209/DC-SIGN, a known binding protein for SARS-CoV and other viruses, as well as multiple inflammatory and thrombotic proteins, while the 3p21.31 locus is associated with levels of CXCL16, a known

inflammatory chemokine. Thus, integration of genetic information and proteomic profiling in biracial cohorts highlights putative mechanisms for genetic risk in COVID-19 disease. [**note: this is another deep dive into the genetic factors related to risk of COVID-19.**] https://www.medrxiv.org/content/10.1101/2020.06.09.20125690v1

- Here, we report the first host genetic study in China by deeply sequencing and analyzing the 332 COVID-19 patients categorized by varying levels of severity from the Shenzhen Third People's Hospital. Based on a total of 22.2 million genetic variants, we conducted both single-variant and gene-based association tests among the five severity groups including asymptomatic, mild, moderate, severe and critical ill patients after the correction of potential confounding factors. The most significant gene loci associated with severity is located in TMEM189-UBE2V1 involved in the IL-1 signaling pathway. The p.Val197Met missense variant that affects the stability of the TMPRSS2 protein displays a decreasing allele frequency among the severe patients compared to the mild and the general population. We also identified that the HLA-A*11:01, B*51:01 and C*14:02 alleles significantly predispose the worst outcome of the patients. This initial study of Chinese patients provides a comprehensive view of the genetic difference among the COVID-19 patient groups and highlighted genes and variants that may help guide targeted efforts in containing the outbreak. Limitations and advantages of the study was also reviewed to guide future international efforts on elucidating the genetic architecture of host-pathogen interaction for COVID-19 and other infectious and complex diseases. [**note: following right next to the previous paper is this study from China on genetic differences among COVID-19 patient groups.**] https://www.medrxiv.org/content/10.1101/2020.06.09.20126607v1
- Tracking the COVID-19 pandemic using existing metrics such as confirmed cases and deaths are insufficient for understanding the trajectory of the pandemic and identifying the next wave of cases. In this study, we demonstrate the utility of monitoring the daily number of patients with COVID-like illness (CLI) who present to the Emergency Department (ED) as a tool that can guide local response efforts. Methods Using data from two hospitals in King County, WA, we examined the daily volume of CLI visits, and compare them to confirmed COVID cases and COVID deaths in the County. A linear regression model with varying lags is used to predict the number of daily COVID deaths from the number of CLI visits. Results CLI visits appear to rise and peak well in advance of both confirmed COVID cases and deaths in King County. Our regression analysis to predict daily deaths with a lagged count of CLI visits in the ED showed that the R2 value was maximized at 14 days. Conclusions ED CLI visits are a leading indicator of the pandemic. Adopting and scaling up a CLI monitoring approach at the local level will provide needed actionable evidence to policy makers and health officials struggling to confront this health challenge. [**note: this may be a useful tool for public health officials to use in assisting analyses of COVID-19 infections.**] https://www.medrxiv.org/content/10.1101/2020.06.09.20126508v1
- Several risk factors have emerged for novel 2019 coronavirus disease (COVID-19) infection and severity. Yet, it is unknown to what degree these risk factors alone or in combination can accurately predict who is most at risk. It is also worthwhile to consider serological antibody titers to non COVID-19 infectious diseases, which may influence host immunity to COVID-19. Methods: In this retrospective study of multicenter UK Biobank participants, as of May 26th 2020, all COVID-19 testing data was collected by Public Health England for older adult in- and out-patients (69.6 ± 8.8 years). We used linear discriminant analysis with cross-validation and bootstrapping to determine the accuracy, specificity, and sensitivity of baseline data from 2006-

2010 to predict COVID-19 infection and presumptive severity (i.e., testing at hospital). Receiver operating characteristic (ROC) curves were used to derive the area under the curve (AUC). Findings: This retrospective study included 4,510 unique participants and 7,539 testing instances (i.e., test cases). Testing resulted in 5,329 negative cases and 2,210 positive cases, split into 996 mild and 1,214 severe disease outcomes. Baseline data including demographics, bioimpedance-derived body composition, vitals, serum biochemistry, self-reported illness/disability, and complete blood count. A randomized subset of 80 participants with 124 test cases also had antibody titers for 20 common to rare infectious diseases. Among all test cases, accuracy was modest for final diagnostic models of COVID-19 infection (70.2%; AUC=0.570, CI=0.556-0.584) and severity (58.3%; AUC=0.592, CI=0.568-0.615). In the sub-group with serology, by contrast, final models predicted infection and severity with an accuracy of 93.5% (AUC=0.969, CI=0.934-1.000) and 74.4% (AUC=0.803, CI=0.663-0.943) respectively. Models included titers to common pathogens (e.g., human cytomegalovirus), age, blood cell counts, lipids, and other biochemical markers. Interpretation: Serological titers for infectious diseases and other risk factors could help policy makers and clinicians better identify who may get COVID-19 and require hospitalization. [**note: I've posted some abstracts on the UK Biobank and here is a another one that goes into a number of markers that might be useful in considering who is at risk for severe COVID-19.**] https://www.medrxiv.org/content/10.1101/2020.06.09.20127092v1

NEWLY REGISTERED CLINICAL TRIALS

- Since its discovery, lactoferrin and its related peptides are mainly considered to be important non-specific host defense molecules against a broad range of viruses including SARS-CoV, which is closely related to SARS-CoV-2 that causes COVID-19. Lactoferrin has been found to experimentally inhibit viral entry in murine coronavirus, and human coronaviruses hCOV-NL63 and pseudotyped SARS-CoV. Besides reducing viral entry, lactoferrin can also suppress virus replication after the viral entry. [**note: this is an Egyptian trial.**] NCT04421534
- The purpose of this study is to assess the safety and efficacy of merimepodib (MMPD) oral solution when administered in combination with remdesivir in adult patients with advanced COVID-19. [**note: sponsored by a company called ViralClear Pharmaceuticals and the trial is at the Mayo Clinic. It's an old drug originally developed by Vertex and never showed any efficacy against Hep C.**] NCT04410354
- This is a multicenter, randomized, double-blind, placebo-controlled phase 2 study of IC14, an antibody to CD14, in reducing the severity of respiratory disease in hospitalized COVID-19 patients. [**note: the trial sponsor is Implicit Biosciences and it is taking place at University of Washington. More is at the company website.**] NCT04391309
- The purpose of this study is to explore the efficacy of Aggrenox in patients with SARS-CoV-2 infection with symptoms consistent with COVID-19. An anticipated total of 132 participants will be randomly divided almost equally into 2 groups: one group will receive Dipyridamole ER 200mg/ Aspirin 25mg orally/enterally along with the standard of care and the other group with receive the standard of care only but no Dipyridamole ER 200mg/ Aspirin 25mg. Participants will be screened, enrolled, receive treatment, and followed for 28 days. The clinical and laboratory outcomes of all the participants enrolled in the study will be evaluated at the end of the study to explore if there is any difference in the outcomes between 2 groups. [**note: trial sponsor is Boehringer Ingelheim.**] NCT04410328

- This is a prospective, phase 2, multicenter, randomized, double blind, placebo controlled, parallel group study to assess the safety and efficacy of CSL312 administered intravenously, in combination with standard of care (SOC) treatment, in patients with Coronavirus disease 2019 (COVID 19) [**note: sponsor is CSL Behring; compound is Garadacimab, Factor XIIa Antagonist Monoclonal Antibody**] NCT04409509

CLINICAL TRIAL RESULTS

- A major dogma in immunology has it that the IgM antibody response precedes secondary memory responses built on the production of IgG, IgA and, occasionaly, IgE. Here, we measured acute humoral responses to SARS-CoV-2, including the frequency of antibody-secreting cells and the presence of specific, neutralizing, antibodies in serum and broncho-alveolar fluid of 145 patients with COVID-19. Surprisingly, early SARS-CoV-2-specific humoral responses were found to be typically dominated by antibodies of the IgA isotype. Peripheral expansion of IgA-plasmablasts with mucosal-homing potential was detected shortly after the onset of symptoms and peaked during the third week of the disease. While the specific antibody response included IgG, IgM and IgA, the latter contributed to a much larger extent to virus neutralization, as compared to IgG. However, specific IgA serum levels notably decrease after one month of evolution. These results represent a challenging observation given the present uncertainty as to which kind of humoral response would optimally protect against re-infection, and whether vaccine regimens should consider boosting a potent, although, at least in blood, fading IgA response. [**note: another intriguing finding about the immune response to SARS-CoV-2 infection. There have been a number of commentaries about long lasting immunity.**] https://www.medrxiv.org/content/10.1101/2020.06.10.20126532v1
- The SARS-CoV-2 outbreak was recently declared a worldwide pandemic. Infection triggers the respiratory tract disease COVID-19, which is accompanied by serious changes of clinical biomarkers such as hemoglobin and interleukins. The same parameters are altered during hemolysis, which is characterized by an increase in labile heme. We present two approaches that aim at analyzing a potential link between available heme and COVID-19 pathogenesis. Four COVID-19 related proteins, i.e. the host cell proteins ACE2 and TMPRSS2 as well as the viral protein 7a and S protein, were identified as potential heme binders. We also performed a detailed analysis of the common pathways induced by heme and SARS-CoV-2 by superimposition of knowledge graphs covering heme biology and COVID-19 pathophysiology. Herein, focus was laid on inflammatory pathways, and distinct biomarkers as the linking elements. Finally, the results substantially improve our understanding of COVID-19 infections and disease progression of patients with different clinical backgrounds and expand the diagnostic and treatment options. [**note: here is another paper on the relationship between heme and COVID-19 pathophysiology.**] https://www.biorxiv.org/content/10.1101/2020.06.09.142125v1

DRUG DEVELOPMENT

- The receptor-binding domain (RBD) of the SARS-CoV-2 spike protein plays a crucial role in binding the human cell receptor ACE2 that is required for viral entry. Many studies have been conducted to target the structures of RBD-ACE2 binding and to design RBD-targeting vaccines and drugs. Nevertheless, mutations distal from the SARS-CoV-2 RBD also impact its

transmissibility and antibody can target non-RBD regions, suggesting the incomplete role of the RBD region in the spike protein-ACE2 binding. Here, in order to elucidate distant binding mechanisms, we analyze complexes of ACE2 with the wild type spike protein and with key mutants via large-scale all-atom explicit solvent molecular dynamics simulations. We find that though distributed approximately 10 nm away from the RBD, the SARS-CoV-2 polybasic cleavage sites enhance, via electrostatic interactions and hydration, the RBD-ACE2 binding affinity. A negatively charged tetrapeptide (GluGluLeuGlu) is then designed to neutralize the positively charged arginine on the polybasic cleavage sites. We find that the tetrapeptide GluGluLeuGlu binds to one of the three polybasic cleavage sites of the SARS-CoV-2 spike protein lessening by 34% the RBD-ACE2 binding strength. This significant binding energy reduction demonstrates the feasibility to neutralize RBD-ACE2 binding by targeting this specific polybasic cleavage site. Our work enhances understanding of the binding mechanism of SARS-CoV-2 to ACE2, which may aid the design of therapeutics for COVID-19 infection. [**note: another possible therapeutic target.**] https://www.biorxiv.org/content/10.1101/2020.06.09.142877v1

- Inspired by fusion-inhibitory peptides from heptad repeat 1 (HR1) and heptad repeat 2 (HR2) domains from human immuno-deficiency virus type 1 (HIV-1) envelope glycoprotein gp41 and severe acute respiratory syndrome-coronavirus (SARS-CoV) based on viral fusogenic mechanism in the present work, we provided a similar approach to design the synthesized peptides against the entry into host cells of SARA-CoV-2 virus that causes 2019 novel coronavirus disease (COVID-19). These peptides derived from HR1 and HR2 of SARA-CoV-2 spike protein were further tested for their interaction and potential fusion possibility through circular dichroism spectrum. Here we used the peptide COVID-2019-HR1P1 (40 amino acids) as the target, which was derived from HR1 of SARA-CoV-2 spike protein, while the designed peptides including COVID-2019-HR2P1 (37 amino acids), COVID-2019-HR2P2 (32 amino acids) and others derived from HR2 of SARA-CoV-2 were tested for their binding to COVID-2019-HR1P1. Interestingly, results showed that both COVID-2019-HR2P1 and COVID-2019-HR2P2 can form the complex with COVID-2019-HR1P1, respectively. This implied that these designed peptides could play an important role in blocking SARA-CoV-2 entry into mammalian host cells via viral fusogenic mechanism, and thus could be used for preventing SARA-CoV-2 infection. [**note: while interesting, getting such long peptides delivered to the site of SARS-CoV-2 infection will be challenging.**] https://www.biorxiv.org/content/10.1101/2020.06.09.142315v1
- SARS-CoV-2, the Covid-19 causative virus, adheres to human cells through binding of its envelope Spike protein to the receptor ACE2. The Spike receptor-binding domain (S-RBD) mediates this key event and thus is a primary target for therapeutic neutralizing antibodies to mask the ACE2-interacting interface. Here, we generated 99 synthetic nanobodies (sybodies) using ribosome and phage display. The best sybody MR3 binds the RBD with KD of 1.0 nM and neutralizes SARS-CoV-2 pseudovirus with IC50 of 0.40 µg/mL. Crystal structures of two sybody-RBD complexes reveal a common neutralizing mechanism through which the RBD-ACE2 interaction is competitively inhibited by sybodies. The structures allowed the rational design of a mutant with higher affinity and improved neutralization efficiency by ≈24-folds, lowering the IC50 from 12.32 to 0.50 µg/mL. Further, the structures explain the selectivity of sybodies between SARS-CoV strains. Our work presents an alternative approach to generate neutralizers against newly emerged viruses. [**note: this is similar to some past studies. Here is a Wikipedia article. While these fragments are easier to produce than mAbs, the question of human**

clearance by the immune system remains. I don't know if anyone has really taken a hard look at this aspect and I haven't seen much on the clinical application of this approach.]
https://www.biorxiv.org/content/10.1101/2020.06.09.143438v1

- Despite the worldwide efforts to avoid disease progression of COVID-19 into a severe acute respiratory syndrome and avoid its severe impact on health systems; the situation remains critical. Effective diagnosis, treatment, and prophylactic measures are required to meet the worldwide demand: recombinant antibodies such as alpaca Nanobodies fulfill these requirements. Here, we develop a fast track for nanobody isolation against the receptor-binding-domain (RBD) SARS-CoV-2 Spike protein following an optimized immunization, efficient construction of the VHH library for E. coli surface display, and single-step selection of high-affinity nanobodies using a simple density gradient centrifugation of the bacterial library. Following this procedure, we isolate and characterize an alpaca Nanobody against Spike RBD of SARS-CoV-2 in the sub-nanomolar range. [**note: here is a Chilean study on isolation of specific nanobodies as per the above paper.**]
https://www.biorxiv.org/content/10.1101/2020.06.09.137935v1

DIAGNOSTIC DEVELOPMENT

- The rapid, accurate and convenient SARS-CoV-2 virus detection is crucial for controlling and stopping the pandemics. Diagnosis of patients in the early stage infection are so far limited to viral nucleic acid or antigen detection in human nasopharyngeal swab or saliva samples. Here we developed a method for rapid and direct optical measurement of SARS-CoV-2 virus particles in one step nearly without any sample preparation using a spike protein specific nanoplasmonic resonance sensor. We demonstrate that we can detect as few as 30 virus particles in one step within 15 minutes and can quantify the virus concentration linearly in the range of 103 vp/ml to 106 vp/ml. Measurements shown on both generic microplate reader and a handheld smartphone connected device suggest that our low-cost and rapid detection method may be adopted quickly under both regular clinical environment and resource-limited settings. [**note: this is a worthwhile paper to read as there are some very good diagrams of how this device would work. Obviously, work so manufacturing and validation are key to any large scale adoption.**] https://www.biorxiv.org/content/10.1101/2020.06.09.142372v1

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2020-06-12

My bassoonist daughter, Jenny, and I saw the Imani Winds about a decade ago when they were in residence at the University of Maryland giving some master classes. She went to one of the classes and we both went to the concert they gave. They are a wonderful group that play an eclectic program of music that includes a number of compositions written for them. Here is Astor Piazzolla's "Libertango": https://www.youtube.com/watch?v=BOMbCsFV4ns that was recorded about a mile up the road from where we live in Bethesda!! Our county was very courageous about 15 years ago when they built a concert hall on the mansion property and we are better for it.

Here is a Washington Post story on the chronic nature of some COVID-19 infections. In the same paper is a wonderful opinion piece by Daniel Oran and Eric Topol of Scripps about asymptomatic individuals and transmission. They note the need for vastly more testing than we are doing right now. There is more by the authors at their Annals of Internal Medicine article; make sure to read the comments that argue against the authors' points.

Derek Lowe offers up a vaccine update.

New York Times article on drugs that calm cytokine storm.

Hydroxychloroquine papers continue to trickle out as you will see below. I'm still curious whether the US trials are fully enrolled.

MODELING

- Between April 6 and May 9, 2020, we enrolled 2766 participants from 1339 households, with a demographic distribution similar to that of the canton of Geneva. In the first week, we estimated a seroprevalence of 4·8% (95% CI 2·4–8·0, n=341). The estimate increased to 8·5% (5·9–11·4, n=469) in the second week, to 10·9% (7·9–14·4, n=577) in the third week, 6·6% (4·3–9·4, n=604) in the fourth week, and 10·8% (8·2–13·9, n=775) in the fifth week. Individuals aged 5–9 years (relative risk [RR] 0·32 [95% CI 0·11–0·63]) and those older than 65 years (RR 0·50 [0·28–0·78]) had a significantly lower risk of being seropositive than those aged 20–49 years. After accounting for the time to seroconversion, we estimated that for every reported confirmed case, there were 11·6 infections in the community. These results suggest that most of the population of Geneva remained uninfected during this wave of the pandemic, despite the high prevalence of COVID-19 in the region (5000 reported clinical cases over <2·5 months in the population of half a million people). Assuming that the presence of IgG antibodies is associated with immunity, these results highlight that the epidemic is far from coming to an end by means of fewer susceptible people in the population. Further, a significantly lower seroprevalence was observed for children aged 5–9 years and adults older than 65 years, compared with those aged 10–64 years. These results will inform countries considering the easing of restrictions aimed at curbing transmission. [**note: this is from a large serology study in Geneva**] https://www.thelancet.com/journals/lancet/article/PIIS0140-6736(20)31304-0/fulltext
- This result is informative for several reasons. First, if herd immunity had been reached because of a large proportion of the population being infected, then one would expect to see a higher seroprevalence and a correspondingly lower slope (equivalent to a lower IFR). The current data in Europe are consistent with an IFR of 0·5–1·0%, which is many times higher than seasonal influenza (<0·1%). Second, if one conjectures that differences between the European countries in our analysis are caused by differences in severity or death reporting, then one would expect to see very different slopes between countries. The data do not support this explanation. Third, if herd immunity has been reached in all regions, then one would expect to see relatively little variation in seroprevalence. Taking Spain as an example, for the country to have achieved herd immunity, one would have to assume that the herd immunity threshold differs by a factor of ten between regions. In contrast, all of these patterns are easily explained if one assumes that interventions are acting to keep deaths and infections at pre-herd immunity levels. This would,

for example, imply that Denmark and Spain have been experiencing a broadly similar IFR but that Denmark has fewer deaths and lower seroprevalence simply because the epidemic did not progress as far as it did in Spain before lockdown came into place. Evidence from outbreaks in confined settings shows the proportion of individuals infected can reach high levels (eg, more than 60%), providing little reason to think the people in these countries who are currently seronegative are not susceptible to infection. [**note: correspondence from the Imperial College group suggesting that no country in Europe has seen infection rates large enough to prevent a second wave of transmission.**] https://www.thelancet.com/journals/lancet/article/PIIS0140-6736(20)31357-X/fulltext

- Background: Following a consistent decline in COVID-19-related deaths in the UK throughout May 2020, it is recognised that contact tracing will be vital to relaxing physical distancing measures. The increasingly evident role of asymptomatic and pre-symptomatic transmission means testing is central to control, but test sensitivity estimates are as low as 65%. Methods: We extend an existing UK-focused branching process model for contact tracing, adding diagnostic testing and refining parameter estimates to demonstrate the impact of poor test sensitivity and suggest mitigation methods. We also investigate the role of super-spreading events, providing estimates of the relationship between infections, cases detected and hospitalisations, and consider how tracing coverage and speed affects outbreak risk. Findings: Incorporating poor sensitivity testing into tracing protocols could reduce efficacy, due to false negative results impacting isolation duration. However, a 7-day isolation period for all negative-testing individuals could mitigate this effect. Similarly, reducing delays to testing following exposure has a negligible impact on the risk of future outbreaks, but could undermine control if negative-testing individuals immediately cease isolating. Even 100% tracing of contacts will miss cases, which could prompt large localised outbreaks if physical distancing measures are relaxed prematurely. Interpretation: It is imperative that test results are interpreted with caution due to high false-negative rates and that contact tracing is used in combination with physical distancing measures. If the risks associated with imperfect test sensitivity are mitigated, we find that contact tracing can facilitate control when the reproduction number with physical distancing, Rs, is less than 15. [**note: I include this model because it discusses the real issue of having imperfect tests and the need for continued physical distancing.**] https://www.medrxiv.org/content/10.1101/2020.06.09.20124008v1
- In January of 2020, COVID-19 became a worldwide pandemic. As many industries shutdown to comply with social distancing measures, the cannabis industry was deemed an essential business in most US jurisdictions. Cannabis is manually farmed, trimmed and packaged and as a result can be a potential inhaled SARs-CoV-2 fomite. Many of the comorbidities described in COVID-19 are also qualifying conditions for medical cannabis access. Bat Guano has been identified as a rich source for novel coronavirus discovery and bat guano is also a common fertilizer in the cannabis field. Employees of cannabis grows have been reported to test qPCR positive for SARs-CoV-2. To better assess cannabis fomite risk we developed a SARs-CoV-2 quantitative PCR assay optimized to operate with a hemp flower background matrix. [**note: this is of interest to one of my loyal readers who has expressed concerns that not enough is being done to collect adverse drug reactions to cannabis. This group seems concerned about impacts on those who grow and cultivate it, in light of the widespread use of bat guano.**] https://www.biorxiv.org/content/10.1101/2020.06.06.112474v1

- The city and county of San Francisco imposed a shelter-in-place order in March 2020, followed by use of a contact tracing program and a policy requiring use of cloth face masks. We used statistical estimation and simulation to estimate the effectiveness of these interventions in San Francisco. We estimated that self-isolation and other practices beginning at the time of San Francisco's shelter-in-place order reduced the effective reproduction number of COVID-19 by 35.4% (95% CI, -20.1%--81.4%). We estimated the effect of contact tracing on the effective reproduction number to be a reduction of approximately 44% times the fraction of cases that are detected, which may be modest if the detection rate is low. We estimated the impact of cloth mask adoption on reproduction number to be approximately 8.6%, and note that the benefit of mask adoption may be substantially greater for essential workers and other vulnerable populations, residents return to circulating outside the home more often. We estimated the effect of those interventions on incidence by simulating counterfactual scenarios in which contact tracing was not adopted, cloth masks were not adopted, and neither contact tracing nor cloth masks was adopted, and found increases in case counts that were modest, but relatively larger than the effects on reproduction numbers. These estimates and model results suggest that testing coverage and timing of testing and contact tracing may be important, and that modest effects on reproduction numbers can nonetheless cause substantial effects on case counts over time. [**note: this is of personal interest as my daughter works in the city at Benioff Children's Hospital.**] https://www.medrxiv.org/content/10.1101/2020.06.09.20125831v1
- Despite social distancing and shelter-in-place policies, COVID-19 continues to spread in the United States. A lack of timely information about factors influencing COVID-19 spread and testing has hampered agile responses to the pandemic. We developed How We Feel, an extensible web and mobile application that aggregates self-reported survey responses, to fill gaps in the collection of COVID-19-related data. How We Feel collects longitudinal and geographically localized information on users' health, behavior, and demographics. Here we report results from over 500,000 users in the United States from April 2, 2020 to May 12, 2020. We show that self- reported surveys can be used to build predictive models of COVID-19 test results, which may aid in identification of likely COVID-19 positive individuals. We find evidence among our users for asymptomatic or presymptomatic presentation, as well as for household and community exposure, occupation, and demographics being strong risk factors for COVID-19. We further reveal factors for which users have been SARS-CoV-2 PCR tested, as well as the temporal dynamics of self- reported symptoms and self-isolation behavior in positive and negative users. These results highlight the utility of collecting a diverse set of symptomatic, demographic, and behavioral self- reported data to fight the COVID-19 pandemic. [**note: this is part of a large effort by The How We Feel Project, a public non-profit group that has set up an app to help out in the tracking and fight against SARS-CoV-2. You too can make a difference!**] https://www.medrxiv.org/content/10.1101/2020.06.09.20126813v1

NEWLY REGISTERED CLINICAL TRIALS

- Will check tomorrow.

CLINICAL TRIAL RESULTS

- The potential benefit of a combination therapy with lopinavir/ritonavir (LPV/r) and hydroxychloroquine (HCQ) on COVID-19 has been speculated. We explored the effect of the

timing of LPV/r + HCQ initiation on the outcome of patients with COVID-19. Methods A retrospective cohort study was conducted on patients with COVID-19 who started treatment with LPV/r plus HCQ between February 21 and March 20, 2020, at Luigi Sacco Hospital in Milan, Italy. Over time cumulative incidence of clinical improvement was compared between patients who started treatment less than 5 days from the onset of symptoms [early treatment group (ET)] and those who initiated it later [delayed treatment group (DT)]. The association of LPV/r plus HCQ initiation timing on 30-day mortality was also assessed by univariate and multivariate logistic models. Results The study included 172 patients, prevalently males (72%) in their sixties, with a moderate (53.4%) or severe (34.9%) disease. Fourty-three (25%) patients were included in the ET group and and 129 (75%) in the DT group. Severity of disease did not significantly differ between the two groups. Conclusion Timing of LPV/r + HCQ initiation seems to have no impact on COVID-19 clinical course in terms of improvement and 30-day mortality. *These findings rise doubts on the clincial efficacy of this regimen*. [**note: from a single Milan hospital looking at the combination treatment of HCQ with lopinavir/ritonavir. 172 patients split between early and later treatment. Timing seems to show not impact of treatment. There is a small meta-analysis of controlled trials that shows pretty much the same thing. Until we have some robust controlled trials on HCQ w/wo any additional agent, the drug should be dropped from standard of care in hospital settings.**]
https://www.medrxiv.org/content/10.1101/2020.06.05.20123299v1

- While several trials are ongoing for treatment of COVID-19, scientific research on chemoprophylaxis is still lacking even though it has potential to delay the pandemic allowing us time to complete research on vaccines. Methods: We have conducted a cohort study amongst Health Care Workers (HCW) exposed to COVID-19 patients, at a tertiary care center in India where there was an abrupt cluster outbreak within on duty personnel. HCWs who had voluntarily taken hydroxychloroquine (HCQ) prior to exposure were considered one cohort while those who had not were considered to be another. All participants with a verifiable contact history were tested for COVID-19 by rtPCR. The two cohorts were comparable in terms of age, gender, comorbidities and exposure. The primary outcome was incidence rates of rtPCR positive COVID-19 infection amongst HCQ users and non - users. Results: 106 healthcare workers were examined in this cohort study of whom 54 were HCQ users and rest were not. The comparative analysis of incidence of infection between the two groups demonstrated that voluntary HCQ usage was associated with lesser likelihood of developing SARS-CoV-2 infection, compared to those who were not on it, $X2=14.59$, $p<0.001$. None of the HCQ users noted any serious adverse effects. Conclusions: This study demonstrated that voluntary HCQ consumption as pre-exposure prophylaxis by HCWs is associated with a statistically significant reduction in risk of SARS-CoV-2. These promising findings therefore highlight the need to examine this association in greater detail among a larger sample using Randomised Controlled Trials (RCT). [**note: this is on pre-exposure use of HCQ and is uncontrolled. The numbers are small and it really is not meaningful. If the Duke study ever gets fully enrolled this will be answered.]**
https://www.medrxiv.org/content/10.1101/2020.06.09.20116806v1
- With the aim of diminishing the impact in Hospital admissions and reducing the number of medical complications, we implemented a strategy based on a Hospital Home-Care Unit (HHCU) using an easy-to-use treatment based on an oral administration regimen outside the hospital with hydroxychloroquine (HCQ) plus azithromycin (AZM) for a short period of 5 days. Patients

and methods: Patients ≥ 18 years old visiting the emergency room at the Hospital Universitario San Pedro de Logrono (La Rioja) between March, 31st and April, 12th diagnosed with COVID-19 with confirmed SARS-CoV-2 infection by a specific PCR, as follows: Patients with pneumonia (CURB ≤ 1) who did not present severe comorbidities and had no processes that contraindicated this therapeutic regime. Olygosimptomatic patients without pneumonia aged ≥ 55 years. Patients ≥ 18 years old without pneumonia with significant comorbidities. We excluded patients with known allergies to some of the antimicrobials used and patients treated with other drugs that increase the QTc or with QTc >450msc. The therapeutic regime was: HCQ 400 mg every twice in a loading dose followed by 200 mg twice for 5 days, plus AZM 500 mg on the first day followed by 250 mg daily for 5 days. A daily telephone follow-up was carried out from the hospital by the same physician. The end-points of our study were: 1.- To measure the need for hospital admission within 15 days after the start of treatment. 2.- To measure the need to be admitted to the intensive care unit (ICU) within 15 days after the start of the treatment. 3.- To describe the severity of the clinical complications developed. 4.- To measure the mortality within 30 days after starting treatment (differentiating if the cause is COVID-19 or something else). 5.-To describe the safety and adverse effects of the therapeutic regime. Results: During the 13 days studied a total of 502 patients were attended in the emergency room due to COVID-19. Forty-two were sent at home; 80 were attended by the HHCU (patients on this study) and 380 were admitted to the Hospital. In our series there were a group of 69 (85.18%) patients diagnosed with pneumonia (37 males and 32 females). Most of them, 57 (82.60%) had a CURB65 score of <1 (average age 49) and 12 (17.40%) a CURB score of 1 (average age 63). Eighteen (22.50%) of the pneumonia patients also had some morbidity as a risk factor. 11 patients (13.75%) without pneumonia were admitted to the HHCU because comorbidities or age ≥ 55 years. Six patients with pneumonia had to be hospitalized during the observation period, 3 of them because side effects and 3 because of worsening. One of these patients, with morbid obesity and asthma, had clinical worsening needing mechanical ventilation at ICU and developed acute distress respiratory syndrome. With the exception of the patient admitted to the ICU, the rest of the patients were discharged at home in the following 8 days (3 to 8 days). Twelve patients (15%), 11 of whom had pneumonia, experienced side effects affecting mainly the digestive. In another patient a QTc interval prolongation (452 msc) was observed. In total 3 of these patients had to be admitted in the Hospital, 2 because of vomiting and 1 because a QTc interval lengthening. None of the patients needed to stop the HCQ or AZM and all the 80 patients finished the therapeutic strategy. From the group without pneumonia only a patient developed diarrhea that did not require hospitalization or stop the medication. Conclusions: Our strategy has been associated with a reduction in the burden of hospital pressure, and it seems to be successful in terms of the number of patients who have developed serious complications and / or death. None of the patients died in the studied period and only 6 have to be admitted in conventional hospitalization area. [**note: yet another uncontrolled study of HCQ/azithromycin. The drug therapy was only given to those patient who had moderate symptoms including pneumonia so the number treated is small. I will repeat once again, TIWWDCT!!!!.**] https://www.medrxiv.org/content/10.1101/2020.06.10.20101105v1

DRUG DEVELOPMENT

- Currently, effective vaccines or specific therapeutic agents against COVID-19 are not available. However, in China, traditional Chinese herbal medicines have provided therapeutic benefit to patients with COVID-19. Jinhua Qinggan granule (JHQGG) is a Chinese multi-herbal formula previously developed for the treatment of H1N1 influenza and has been encouraged for patients clinically suspected of COVID-19 during medical observation. However, the immunological mechanism for the efficacy of JHQGG has not been confirmed. Objectives: We thus examined whether the administration of JHQGG affects hematological and immunological measures in healthy individuals. Method: We enrolled 18 healthy volunteers, all of whom tested negative for antibodies to SARS-CoV-2. Peripheral blood samples were collected 1 h after oral administration of JHQGG and subjected to hematological, biochemical, and cytokine tests. Results: JHQGG rapidly induced a significant decrease in the plasma level of IL-6 and an increase in the plasma level of IFN-γ. Conclusions: Our finding suggests that the therapeutic efficacy of JHQGG against COVID-19 is, in part, associated with its rapid immunomodulatory activity.[**note: this article is from Japan, not China. China still uses a lot of traditional herbal medicines in clinics. I've not seen any controlled trial results for this particular preparation.**]
  https://www.medrxiv.org/content/10.1101/2020.06.08.20124453v1
- Here we assessed the prophylactic/therapeutic efficacy of hydroxychloroquine (HCQ), a drug of interest for COVID-19 management, in two animal models. When used for prophylaxis or treatment neither the standard human malaria dose (6.5 mg/kg) nor a high dose (50 mg/kg) of HCQ had any beneficial effect on clinical disease or SARS-CoV-2 kinetics (replication/shedding) in the Syrian hamster disease model. Similarly, HCQ prophylaxis/treatment (6.5 mg/kg) did not significantly benefit clinical outcome nor reduce SARS-CoV-2 replication/shedding in the upper and lower respiratory tract in the rhesus macaque disease model. In conclusion, our preclinical animal studies do not support the use of HCQ in prophylaxis/treatment of COVID-19. [**note: since these animal models are used for drug and vaccine development it seems pertinent to note that HCQ does not work in them. Of course they are only animal models and maybe the secret sauce of zinc, Vitamin D, and azithromycin are needed for therapeutic activity.**]
  https://www.biorxiv.org/content/10.1101/2020.06.10.145144v1
- A SARS-CoV-2 vaccine is needed to control the global COVID-19 public health crisis. Atomic-level structures directed the application of prefusion-stabilizing mutations that improved expression and immunogenicity of betacoronavirus spike proteins. Using this established immunogen design, the release of SARS-CoV-2 sequences triggered immediate rapid manufacturing of an mRNA vaccine expressing the prefusion-stabilized SARS-CoV-2 spike trimer (mRNA-1273). Here, we show that mRNA-1273 induces both potent neutralizing antibody and CD8 T cell responses and protects against SARS-CoV-2 infection in lungs and noses of mice without evidence of immunopathology. mRNA-1273 is currently in a Phase 2 clinical trial with a trajectory towards Phase 3 efficacy evaluation. [**note: from a large group of researchers, mainly from NIAID. This is the Moderna vaccine.**] https://www.biorxiv.org/content/10.1101/2020.06.11.145920v1
- In the light of the urgent need to identify novel approaches to be used in the emergency phase, a largely explored strategy has been the repurpose of clinically available drugs as new antivirals, by targeting different viral proteins. In this paper, we describe a drug repurposing strategy based on a virtual screening of druggable pockets located in the central β-sheet core of the SARS-CoV-2 Spike protein RBD supported by in vitro tests identifying several steroidal derivatives as SARS-CoV-2 entry inhibitors. Our results demonstrate that several potential

binding sites exist in the SARS CoV-2 S protein, and that the occupancy of these pockets reduces the ability of the S protein RBD to bind to the ACE2 consensus in vitro. In particular, natural occurring and clinically available steroids as glycyrrhetinic and oleanolic acids, as well as the bile acids derivatives glyco-UDCA and obeticholic acid have been shown to be effective in preventing virus entry in the case of low viral load. All together, these results might help to define novel approaches to reduce the viral load by using SARS-CoV-2 entry inhibitors. [**note: from Italy, another therapeutic target, this time to block viral entry.**]
https://www.biorxiv.org/content/10.1101/2020.06.10.144964v1

- Antibody development efforts mainly revolve around the extensively glycosylated SARS-CoV-2 spike (S) protein, which mediates the host cell entry by binding to the angiotensin-converting enzyme 2 (ACE2). In the context of vaccine design, similar to many other viruses, the SARS-CoV-2 spike utilizes a glycan shield to thwart the host immune response. Here, we built a full-length model of glycosylated SARS-CoV-2 S protein, both in the open and closed states, augmenting the available structural and biological data. Multiple microsecond-long, all-atom molecular dynamics simulations were used to provide an atomistic perspective on the glycan shield and the protein structure, stability, and dynamics. End-to-end accessibility analyses outline a complete overview of the vulnerabilities of the glycan shield of SARS-CoV-2 S protein, which can be harnessed for vaccine development. In addition, a dynamic analysis of the main antibody epitopes is provided. Finally, beyond shielding, a possible structural role of N-glycans at N165 and N234 is hypothesized to modulate and stabilize the conformational dynamics of the spike's receptor binding domain, which is responsible for ACE2 recognition. Overall, this work presents hitherto unseen functional and structural insights into the SARS-CoV-2 S protein and its glycan coat, which may be exploited by therapeutic efforts targeting this essential molecular machine. [**note: interesting work on the glycan coat of the viral spike protein.**]
https://www.biorxiv.org/content/10.1101/2020.06.11.146522v1
- Gastrointestinal symptoms in COVID-19 are associated with prolonged symptoms and increased severity. We employed human intestinal organoids derived from pluripotent stem cells (PSC-HIOs) to analyze SARS-CoV-2 pathogenesis and to validate efficacy of specific drugs in the gut. Certain, but not all cell types in PSC-HIOs express SARS-CoV-2 entry factors ACE2 and TMPRSS2, rendering them susceptible to SARS-CoV-2 infection. Remdesivir, a promising drug to treat COVID-19, effectively suppressed SARS-CoV-2 infection of PSC-HIOs. In contrast, the histamine-2-blocker famotidine showed no effect. Thus, PSC-HIOs provide an interesting platform to study SARS-CoV-2 infection and to identify or validate drugs. [**note: a new cell model for looking at infection and drug development. They tested this out with both remdesivir and famotidine. The former inhibited viral infection but not the latter.**]
https://www.biorxiv.org/content/10.1101/2020.06.10.144816v1

DIAGNOSTIC DEVELOPMENT

- Supply chain disruptions and scarce availability of commercial laboratory reagents have motivated worldwide actors to search for alternative workflows to cope with the demand. We have used open-source liquid-handling robots (OT2), RNA extraction and RT-qPCR reagents to set-up a reproducible workflow for RT-qPCR COVID-19 testing. We developed a framework with a template and several functions and classes that allow the creation of customized RT-qPCR automated circuits. As a proof of concept, we provide data obtained by a fully-functional tested

code using the MagMax Pathogen RNA/DNA kit (Thermo Fisher Scientific) on the Kingfisher Flex instrument (Thermo Fisher Scientific). With these codes available is easy to create new stations or circuits from scratch, adapt existing ones to changes in the experimental protocol, or perform fine adjustments to fulfil special needs. The affordability of this platform makes it accessible for most laboratories and hospitals with a bioinformatician, democratising automated covid19 PCR testing and increasing, temporarily or not, the capacity of carrying out tests. It also confers flexibility, as this platform is not dependant on any particular commercial kit and can be quickly adapted to protocol changes or other special needs. [**note: pretty cool stuff from Barcelona, an open source flexible diagnostic platform.**]
https://www.biorxiv.org/content/10.1101/2020.06.11.140285v2

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2020-06-13

How about another piano concerto by a Russian composer? Why not? Let's have a listen to the famous Tchaikovsky Piano Concerto #1, admirably played by Anna Federova accompanied by the Nordwestdeutsche Philharmonie led by Yves Abel: https://www.youtube.com/watch?v=hNfpMRSCFPE Here is a nice short video of Ms. Federova discussing Rachmaninoff. **Contest Alert!!! I desperately want to give away a prize. This one should be easy. Who won the inaugural Tchaikovsky Competition in the piano category?**

The New York Times has a story on a mutation that may or may not make SARS-CoV-2 more problematic to control. The finding is from Scripps researchers though there have been other reports on this that I have posed. Preprint is HERE.

I have been reading Bill McBride's *Calculated Risk* blog for just over 10 years. We have corresponded on occasion and I found out that he grew up right near me in San Diego. He was four years behind me in school, so we didn't know one another. He is one of the few who correctly identified the housing bust that led to the 2008 meltdown. He has a great piece on why people may be resistant to wearing masks.

One of the earliest documented outbreaks of COVID-19 was aboard the cruise ship *Diamond Prnicess*. The Lancet has two articles on different aspects of the outbreak HERE and HERE.

The Washington Post has an article on repurposing old vaccines to ward off SARS-CoV-2. Certainly there are trials of BCG going on in several countries. I have not seen much about whether the polio vaccine is useful in this situation. This paper from Tulane researchers may explain how BCG vaccine works against SARS-CoV-2. The clinical trials will inform us one way or the other.

MODELING

- Here we quantify economic and health risk tradeoffs of reopening by industry for each state in the US. To estimate total economic impact, we summed income loss due to unemployment and profit loss. We assess transmission risk by: (1) workplace size, (2) human interactions, (3) inability to work from home, and (4) industry size. We found that the industry with the highest estimated economic impact from COVID-19 was manufacturing in 40 states; the industry with the largest transmission risk index was accommodation and food services in 41 states, and the

industry with the highest economic impact per unit of transmission risk, interpreted as the value of reopening, was manufacturing in 37 states. Researchers and decision makers must work together to consider both health and economics when making tough decisions. [**note: this is a very useful paper to read as they analyze the economic and transmission factors by industrial sector. It is short and to the point.**]
https://www.medrxiv.org/content/10.1101/2020.06.11.20128918v1

- We examined the associations between plasma concentrations of soluble ACE2 and biomarkers of Metabolic Syndrome in a large (N=2,051) sample of individuals who participated in a commercial wellness program and who underwent deep molecular phenotyping. sACE2 levels were significantly higher in men, compared to women, and in individuals with Metabolic Syndrome, compared to controls. sACE2 levels showed reliable associations with all individuals components of Metabolic Syndrome, including obesity, hypertension, insulin resistance, hyperlipidemia, and as well as markers of liver damage. This profile of associations was statistically significantly stronger in men, compared to women, and suggests that preexisting cardiometabolic conditions might confer increased severity of symptoms in some COVID-19 patients through increased expression of ACE2 in the liver. [**note: not clinical data but perhaps a linkage to severe progression.**]
https://www.medrxiv.org/content/10.1101/2020.06.10.20127969v1

NEWLY REGISTERED CLINICAL TRIALS

- Regeneron monoclonal antibody trial [**note: they hope to enroll 1860 patients]** NCT04426695
- This will be a phase 1a randomized, open label, multi-center study with approximately 24 subjects. All subjects will receive standard of care (SOC) per institutional guidelines for treatment of hospitalized patients with COVID-19 infection. In addition to SOC, the brequinar group will receive 5 daily doses of brequinar 100 mg. [**note: this drug has never been approved because of narrow therapeutic index and safety concerns. Sponsor is Clear Creek Bio, a company new to me.**] NCT04425252
- This is a randomized, double-blind, placebo-controlled, in which one dose of nangibotide will be tested versus placebo. [**note: I don't know much about this experimental drug. It's listed as** a TREM-1 (triggering receptor expressed on myeloid cells-1) **inhibitor, is a chemically synthesized peptide that acts as a decoy receptor interfering with the binding of TREM-1 and its ligand. Based on preclinical studies, the Company believes nangibotide will be able to restore a balanced inflammatory response, vascular function, and improve overall survival. Sponsor is Inotrem.**] NCT04429334
- The purpose of this trial is to investigate the efficacy and safety of continuous intravenous administration of low dose iloprost versus placebo for 72-hours, in 80 patients with COVID-19 suffering from respiratory failure. The study hypothesis is that iloprost may be beneficial as an endothelial rescue treatment as it is anticipated to deactivate the endothelium and restore vascular integrity in COVID-19 patients suffering from respiratory failure caused by endothelial breakdown, ultimately improving survival. Given that the pulmonary system, apart from the brain, is the most highly vascularized vital organ in the body, extensive endothelial damage is a central feature of acute respiratory distress syndrome (ARDS) with respiratory failure being the rationale for the current study COMBAT-COVID-19. [**note: this is a Danish trial of a drug used**

for pulmonary hypertension, scleoderma and Raynaud's phenomenon (which I get during the winter months!!!)] NCT04420741

- To evaluate the efficacy of intravenous LSALT peptide plus standard of care to prevent the progression of COVID-19 to mild, moderate or severe ARDS, acute kidney injury, cardiomyopathy, acute liver injury, coagulopathy, or death in patients infected with SARS-CoV-2 compared with placebo plus standard of care. [**note: sponsor is Arch Biopartners and more information is at their website.**] NCT04402957
- The study aims to evaluate MN-166 (ibudilast) in patients with COVID-19 who are at risk of developing acute respiratory distress syndrome. Subjects will be screened, randomly assigned to MN-166 or placebo groups, receive study drug on Days 1-7, and followed up on Day 14 and Day 28. [**note: drug is a phosphodiesterase inhibitor and approved for use in Japan. Sponsor is MedicNova.**] NCT04429555
- GC004 is a Phase I trial to evaluate the safety and the immune responses of a therapeutic vaccine in SARS-CoV-2 infected patients. Covid-19 confirmed patients with mild or no symptoms will be enrolled sequentially into low dose and high dose groups. Following the vaccination subjects who received at least one vaccination will be followed for safety through week 26. [**note: another vaccine I don't know much about. The sponsor is Genecure Biotechnologies, but the vaccine looks like it comes from an Australian group.**] NCT04428073

CLINICAL TRIAL RESULTS

- Angiotensin converting enzyme inhibitors (ACEs) and angiotensin receptor blockers (ARBs) could influence infection risk of coronavirus disease (COVID-19). Observational studies to date lack pre-specification, transparency, rigorous ascertainment adjustment and international generalizability, with contradictory results. Methods: Using electronic health records from Spain (SIDIAP) and the United States (Columbia University Irving Medical Center and Department of Veterans Affairs), we conducted a systematic cohort study with prevalent ACE, ARB, calcium channel blocker (CCB) and thiazide diuretic (THZ) use to determine relative risk of COVID-19 diagnosis and related hospitalization outcomes. The study addressed confounding through large-scale propensity score adjustment and negative control experiments. Results: Following over 1.1 million antihypertensive users identified between November 2019 and January 2020, we observed no significant difference in relative COVID-19 diagnosis risk comparing ACE/ARB vs CCB/THZ monotherapy (hazard ratio: 0.98; 95% CI 0.84 - 1.14), nor any difference for mono/combination use (1.01; 0.90 - 1.15). ACE alone and ARB alone similarly showed no relative risk difference when compared to CCB/THZ monotherapy or mono/combination use. Directly comparing ACE vs. ARB demonstrated a moderately lower risk with ACE, non-significant for monotherapy (0.85; 0.69 - 1.05) and marginally significant for mono/combination users (0.88; 0.79 - 0.99). We observed, however, no significant difference between drug- classes for COVID-19 hospitalization or pneumonia risk across all comparisons. Conclusion: There is no clinically significant increased risk of COVID-19 diagnosis or hospitalization with ACE or ARB use. Users should not discontinue or change their treatment to avoid COVID-19. [**note: this is a large observational study from the OHDSI group.**]
  https://www.medrxiv.org/content/10.1101/2020.06.11.20125849v1
- Severe complications are observed only in a small proportion of infected patients but the cellular mechanisms underlying this progression are still unknown. Comprehensive flow

cytometry of whole blood samples from 54 COVID-19 patients revealed a dramatic increase in the number of immature neutrophils. This increase strongly correlated with disease severity and was associated with elevated IL-6 and IP-10 levels, two key players in the cytokine storm. The most pronounced decrease in cell counts was observed for CD8 T-cells and VD2 gd T-cells, which both exhibited increased differentiation and activation. ROC analysis revealed that the count ratio of immature neutrophils to CD8 or VD2 T-cells predicts pneumonia onset (0.9071) as well as hypoxia onset (0.8908) with high sensitivity and specificity. It would thus be a useful prognostic marker for preventive patient management and improved healthcare resource management. [**note: more biomarker information related to severe COVID-19.**]
https://www.biorxiv.org/content/10.1101/2020.06.11.147389v1

- Eighty nine patients with COVID-19 pneumonia and heightened systemic inflammation (elevated serum C reactive protein and interleukin-6 levels) were treated with Tocilizumab (TCZ), a human monoclonal IgG1 antibody to the interleukin-6 receptor. Results: Clinical and laboratory improvement was seen comparing baseline and 1-2 day post-infusion indices. Among 72 patients not receiving mechanical ventilation, NEWS2 scores fell from 5 to 2 ($p < 0.001$) C reactive protein levels fell from 95 to 14 mg/L ($p < 0.001$) and lymphocyte counts rose from 900 to 1000/uL ($p = 0.036$). Sixty three of 72 patients were discharged from hospital, one patient died, and 8 remained in hospital at time of writing. Among 17 patients receiving mechanical ventilation, despite a rapid decrease in CRP levels from 89 to 35 mg/L ($p = 0.014$) and early improvements in NEWS2 scores in 10 of 17, ten patients died and seven remain in hospital at time of writing. Overall, mortality was only seen in patients who had markedly elevated CRP levels (>30 mg/L) and low lymphocyte counts (<1000/uL) before TCZ administration. Conclusions: Inflammation and lymphocytopenia are linked to mortality in COVID-19. Inhibition of IL-6 activity by administration of Tocilizumab, an anti IL-6 receptor antibody is associated with rapid improvement in both CRP and lymphocyte counts and in clinical indices. Controlled clinical trials are needed to confirm the utility of IL-6 blockade in this setting. Additional interventions will be needed for patients requiring mechanical ventilation. [**note: more data on tocilizumab, this time from Russia. The evidence is increase about its utility. With all the clinical trials going on, we still have not seen a DSMB step in to inform the medical community that this needs to be standard of care.**]
https://www.medrxiv.org/content/10.1101/2020.06.12.20122374v1
- However, to accurately describe any underlying pathophysiology with longitudinal data, the individual patient trajectories have to be synchronized based on temporal markers. In this study, we use longitudinal data from 28 critically ill ICU COVID-19 patients to compare the commonly used alignment markers "onset of symptoms", "hospital admission" and "ICU admission" with a novel objective method based on the peak value of inflammatory marker C-reactive protein (CRP). By applying our CRP-based method to align the progression of neutrophils and lymphocytes, we were able to define a pathophysiological window that allowed further mortality risk stratification in our COVID-19 patient cohort. Our data highlights that proper synchronization of patient data to the underlying pathophysiology is crucial to differentiate severity subgroups and to allow reliable interpatient comparisons. [**note: from Switzerland, looking at C-reactive protein as a marker for COVID-19 severity progression. Interestingly, the Russian paper above notes that tocilizumab brings about a reduction in this marker.**]
https://www.medrxiv.org/content/10.1101/2020.06.11.20128041v1

- In COVID-19, high levels of granulocyte macrophage-colony stimulating factor (GM-CSF) and inflammatory myeloid cells correlate with disease severity, cytokine storm, and respiratory failure. With this rationale, we used lenzilumab, an anti-human GM-CSF monoclonal antibody, to treat patients with severe COVID-19 pneumonia. Methods: Hospitalized patients with COVID-19 pneumonia and risk factors for poor outcomes were treated with lenzilumab 600 mg intravenously for three doses through an emergency single-use IND application. Patient characteristics, clinical and laboratory outcomes, and adverse events were recorded. All patients receiving lenzilumab through May 1, 2020 were included in this report. Results: Twelve patients were treated with lenzilumab. Clinical improvement was observed in 11 out of 12 (92%), with a median time to discharge of 5 days. There was a significant improvement in oxygenation: The proportion of patients with SpO2/FiO2 < 315 at the end of observation was 8% vs. compared to 67% at baseline (p=0.00015). A significant improvement in mean CRP and IL-6 values on day 3 following lenzilumab administration was also observed (137.3 mg/L vs 51.2 mg/L, p = 0.040; 26.8 pg/mL vs 16.1 pg/mL, p = 0.035; respectively). Cytokine analysis showed a reduction in inflammatory myeloid cells two days after lenzilumab treatment. There were no treatment-emergent adverse events attributable to lenzilumab, and no mortality in this cohort of patients with severe COVID-19 pneumonia. Conclusions: In high-risk COVID-19 patients with severe pneumonia, GM-CSF neutralization with lenzilumab was safe and associated with improved clinical outcomes, oxygen requirement, and cytokine storm. [**note: these are early results from a registered Mayo Clinic trial on lenzilumab. Another drug to add to the arsenal though we do need to see how the completed trial turns out.**]
  https://www.medrxiv.org/content/10.1101/2020.06.08.20125369v1

DRUG DEVELOPMENT

- To identify SARS-CoV-2 neutralizing antibodies, we analysed the antibody response of 12 COVID-19 patients from 8 to 69 days post diagnosis. By screening 4,313 SARS-CoV-2-reactive B cells, we isolated 255 antibodies from different time points as early as 8 days post diagnosis. Among these, 28 potently neutralized authentic SARS-CoV-2 (IC100 as low as 0.04 µg/ml), showing a broad spectrum of V genes and low levels of somatic mutations. Interestingly, potential precursors were identified in naive B cell repertoires from 48 healthy individuals that were sampled before the COVID-19 pandemic. *Our results demonstrate that SARS-CoV-2 neutralizing antibodies are readily generated from a diverse pool of precursors, fostering the hope of rapid induction of a protective immune response upon vaccination*. [**note: I never quite know where to put these antibody study papers. Since this one mentions the correlation to vaccine development it goes here. The finding of potential precursors in some of the pre-pandemic samples is intriguing and perhaps explains why some people don't progress to serious COVID-19**] https://www.biorxiv.org/content/10.1101/2020.06.12.146290v1
- Here, we determined the X-ray crystal structure of a potent neutralizing monoclonal antibody, CV30, isolated from a patient infected with SARS-CoV-2, in complex with the receptor binding domain (RBD). The structure reveals CV30's epitope overlaps with the human ACE2 receptor binding site thus providing the structural basis for its neutralization by preventing ACE2 binding. [**note: from Seattle, the crystal structure of a potent monoclonal antibody.**]
  https://www.biorxiv.org/content/10.1101/2020.06.12.148692v1

- In this study, we show that the SARS-CoV-2 nucleocapsid protein (N-protein) undergoes liquid-liquid phase separation (LLPS) with the viral genome, and propose a model of viral packaging through LLPS. N-protein condenses with specific RNA sequences in the first 1000 nts (5'-End) under physiological conditions and is enhanced at human upper airway temperatures. N-protein condensates exclude non-packaged RNA sequences. We comprehensively map sites bound by N-protein in the 5'-End and find preferences for single-stranded RNA flanked by stable structured elements. Liquid-like N-protein condensates form in mammalian cells in a concentration-dependent manner and can be altered by small molecules. Condensation of N-protein is sequence and structure specific, sensitive to human body temperature, and manipulatable with small molecules thus presenting screenable processes for identifying antiviral compounds effective against SARS-CoV-2. [**note: you need to read the paper to get a better understanding of what the authors are looking at. It looks like this approach may block viral assembly by disrupting N-protein condensates. Cyclosporine was one compound they looked at.**] https://www.biorxiv.org/content/10.1101/2020.06.11.147199v1

DIAGNOSTIC DEVELOPMENT

- To minimize the risk of exposure during testing, reduce personal protective equipment use, and increase access to testing, we compared the diagnostic equivalence of a modified specimen collection method, patient-collected lower nasal swabs, with that of the current clinical standard, health care worker–collected oropharyngeal swabs. If the 2 methods proved to be diagnostically equivalent, patients would be able to collect specimens themselves without exposing health care workers to respiratory secretions. Self-collected lower nasal swabs could also be used for home- or office-based testing of asymptomatic patients. However, these preliminary findings are limited by small sample size, have limited generalizability, and need to be validated further in diverse clinical settings. These validation efforts are currently under way at our institution. [**note: Here is a Stanford study of patient collected nasal swabs. The authors note that it is a small study but it does point to the utility of this approach**.] https://jamanetwork.com/journals/jamanetworkopen/fullarticle/2767065
- Quantitative suspension array technology (qSAT) assays based on the xMAP Luminex platform overcome the limitations of rapid diagnostic tests and ELISA with their higher precision, dynamic range, throughput, miniaturization, cost-efficacy and multiplexing capacity. We developed three qSAT assays to detect IgM, IgA and IgG to a panel of eight SARS-CoV-2 antigens including spike (S), nucleoprotein (N) and membrane (M) protein constructs. The assays were optimized to minimize processing time and maximize signal to noise ratio. We evaluated the performance of the assays using 128 plasmas obtained before the COVID-19 pandemic (negative controls) and 115 plasmas from individuals with SARS-CoV-2 diagnosis (positive controls), of whom 8 were asymptomatic, 58 had mild symptoms and 49 were hospitalized. Pre-existing IgG antibodies recognizing N, M and S2 proteins were detected in negative controls suggestive of cross-reactive to common cold coronaviruses. The best performing antibody isotype/antigen signatures had specificities of 100% and sensitivities of 94.94% at ≥14 days since the onset of symptoms and 96.08% at ≥21 days since the onset of symptoms, with AUC of 0.992 and 0.999, respectively. Combining multiple antibody markers as assessed by qSAT assays has the highest efficiency, breadth and versatility to accurately detect low-level antibody responses for obtaining reliable data on prevalence of exposure to novel pathogens in a population. Our assays will allow gaining

insights into antibody correlates of immunity required for vaccine development to combat pandemics like the COVID-19. [**note: from Spain, a multiplex antibody platform that is useful in detecting low level antibody response.**]
https://www.biorxiv.org/content/10.1101/2020.06.11.147363v1

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2020-06-14

For reflection Sunday we have Bach's Goldberg Variations. This is a special version, performed by a number of instrumentalists (and some vocalists) for these pandemic times: https://www.youtube.com/watch?v=O2QqWC7CKeI I'll say no more other than to urge you to listen and think.

The Washington Post has a story about how masks can help in the fight against COVID-19. Of course this is something that all my loyal readers know and practice on a daily basis when out in public. As I read through the paper I was struck by a debate over a Proceedings of the National Academy of Science paper by a group of atmospheric scientists at Texas A&M who posit that wearing masks is the single best thing to prevent the spread of COVID-19. The paper was submitted by Nobel Laureate Mario Molina. This elicited a Twitter storm of adverse comments about the paper suggesting it be retracted. Haven't these people something better to do (this is yet another example as to why nobody should have a Twitter account)? Oh, I forgot they are academics and hence subject to Sayre's Law.

Unfortunately, we are seeing a spike in COVID-19 cases in a number of states.

While I have offered daily musical selections, I've not commented much on what to stream as there are so many choices. As a maven of popular culture I do track what the famous and near famous are watching (number one on the list seems to be the ABC series 'Lost' that I watched in real time when it surfaced over a decade ago). I came across this New York Times appreciation of the short Amazon Prime series 'Red Oaks' that I can also recommend for it's humor and good spirit. For the really ambitious viewer, no recommendation comes higher than David Simon's analysis of the Baltimore drug trade and associated problems in HBO's 'The Wire' that in my humble opinion is the greatest television series ever made. Take it one episode per day and resist the urge to binge watch.

Today was mercifully short for abstract/paper reading. There is an intriguing paper from South Korea on the possible protective nature of ARB/ACEI class of drugs.

MODELING

- As Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) spreads around the World, many questions about the disease are being answered; however, many more remain poorly

understood. Although the situation is rapidly evolving, with datasets being continually corrected or updated, it is crucial to understand what factors may be driving transmission through different populations. While studies are beginning to highlight specific parameters that may be playing a role, few have attempted to thoroughly estimate the relative importance of these disparate variables that likely include: climate, population demographics, and imposed state interventions. In this report, we compiled a database of more than 28 potentially explanatory variables for each of the 50 U.S. states through early May 2020. Using a combination of traditional statistical and modern machine learning approaches, we identified those variables that were the most statistically significant, and, those that were the most important. These variables were chosen to be fiduciaries of a range of possible drivers for COVID-19 deaths in the USA. We found that population-weighted density (PWD), some "stay at home" metrics, monthly temperature and precipitation, race/ethnicity, and chronic low respiratory death rate, were all statistically significant. Of these, PWD and mobility metrics dominated. This suggests that the biggest impact on COVID-19 deaths was, at least initially, a function of where you lived, and not what you did. However, clearly, increasing social distancing has the net effect of (at least temporarily) reducing the effective PWD. Our results strongly support the idea that the loosening of "lock-down" orders should be tailored to the local PWD. In contrast to these variables, while still statistically significant, race/ethnicity, health, and climate effects could only account for a few percent of the variability in deaths. Where associations were anticipated but were not found, we discuss how limitations in the parameters chosen may mask a contribution that might otherwise be present. [**note: this model comes from a small San Diego company that has past expertise in disease modeling. It's interesting in that they try to look across as many available data sets and have an honest discussion of the limitations of each and why some results might be confounding. It is an easy to read paper.**]
https://www.medrxiv.org/content/10.1101/2020.06.11.20129007v1

- Several studies indicated that children seem to be less frequently infected with SARS-CoV-2 and potentially less contagious. To examine the spread of SARS-CoV-2 we combined both RT-PCR testing and serology in children in the most affected region in France, during the COVID-19 epidemic. Methods. From April 14, 2020 to May 12, 2020, we conducted a cross-sectional prospective, multicenter study. Healthy controls and pauci-symptomatic children from birth to age 15 years were enrolled by 27 ambulatory pediatricians. A nasopharyngeal swab was taken for detection of SARS-CoV-2 by RT-PCR and a microsample of blood for micro-method serology. Results. Among the 605 children, 322 (53.2%) were asymptomatic and 283 (46.8%) symptomatic. RT-PCR testing and serology were positive for 11 (1.8%) and 65 (10.7%) of all children, respectively. Only 3 children were RT-PCR-positive without any antibody response have been detected. The frequency of positivity on RT-PCR for SARS-CoV-2 was significantly higher in children with positive serology than those with a negative one (12.3% vs 0.6%, p<0.001). Contact with a person with proven COVID-19 increased the odds of positivity on RT-PCR (OR 7.8, 95% confidence interval [1.5; 40.7]) and serology (15.1 [6.6; 34.6]). Conclusion. *In area heavily affected by COVID-19, after the peak of the first epidemic wave and during the lockdown, the rate of children with positive SARS-CoV-2 RT-PCR was very low (1.8%), but the rate of positive on serology was higher (10.7%). Most of PCR positive children had at the same time, positive serology suggesting a low risk of transmission*. [**note: data on the infection rate in children in a**

hard it French community shows a low rate of infection.]
https://www.medrxiv.org/content/10.1101/2020.06.12.20129221v1

- The necessity of keeping open and accessible public commercial establishments such as supermarkets or pharmacies increases during the pandemic provided that distancing rules and crowd control are satisfied. Herein, using agent-based models, we explore the potential spread of the novel SARS-CoV-2 considering the case of a small size supermarket. For diverse distancing rules and number of simultaneous users (customers), we question flexible and limited movement policies, guiding the flow and interactions of users in place. Results indicate that a guided, limited in movement and well-organized policy combined with a distance rule of at least 1 m between users and a small number of them (15) may aid in the mitigation of potential new contagions in more than 90% compared to the usual policy of flexible movement with more users (30) which may reach up to 64% of mitigation of potential new infections under the same distancing conditions. This study may guide novel strategies for the mitigation of the current COVID-19 pandemic, at any stage, and prevention of future outbreaks of SARS-CoV-2 or related viruses. [**note: the title of this paper, "*When is SARS-CoV-2 in your shopping list?*" guarantees a mention in the newsletter. I'm the chosen one who does the grocery shopping and it's my experience that the stores I go to are practicing what is outlined in this paper.**]
https://www.medrxiv.org/content/10.1101/2020.06.11.20128850v1
- SARS-CoV-2 was detected in Barcelona sewage long before the declaration of the first COVID-19 case, indicating that the infection was present in the population before the first imported case was reported. Sentinel surveillance of SARS-CoV-2 in wastewater would enable adoption of immediate measures in the event of future COVID-19 waves. [**note: I'm curious whether any US communities are doing this type of surveillance.**]
https://www.medrxiv.org/content/10.1101/2020.06.13.20129627v1
- Given the higher mortality rate and widespread phenomenon of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS CoV-2) within the United States (US) population, understanding the mutational pattern of SARS CoV-2 has global implications for detection and therapy to prevent further escalation. Los Angeles has become an epicenter of the SARS-CoV-2 pandemic in the US. Efforts to contain the spread of SARS-CoV-2 require identifying its genetic and geographic variation and understanding the drivers of these differences. For the first time, we report genetic characterization of SARS-CoV-2 genome isolates in the Los Angeles population using targeted next generation sequencing (NGS). Samples collected at Cedars Sinai Medical Center were collected from patients with confirmed SARS-CoV-2 infection. We identified and diagnosed 192 patients by our in-house qPCR assay. In this population, the highest frequency variants were in known mutations in the 5'UTR, AA193 protein, RdRp and the spike glycoprotein. SARS-CoV-2 transmission within the local community was tracked by integrating mutation data with patient postal codes with two predominant community spread clusters being identified. Notably, significant viral genomic diversity was identified. Less than 10 percent of the Los Angeles community samples resembled published mutational profiles of SARS-CoV-2 genomes from China, while >50 percent of the isolates shared closely similarities to those from New York State. Based on these findings we conclude SARS-CoV-2 was likely introduced into the Los Angeles community predominantly from New York State but also via multiple other independent transmission routes including but not limited to Washington State and China. [**note: ain't molecular epidemiology grand? Predominant rate of infection in Los Angeles came from the**

SARS-CoV-2 genotype introduced into the US at the New York City port of entry.]
https://www.medrxiv.org/content/10.1101/2020.06.12.20129999v1

NEWLY REGISTERED CLINICAL TRIALS

- Will check tomorrow.

CLINICAL TRIAL RESULTS

- COVID-19 predisposes to venous thromboembolism and there are multiple data regarding high incidence of venous thrombosis in critical COVID-19 patients, however reports on this complication in less severe patients are not widely available. The aim of this study was to investigate the incidence of deep-vein thrombosis (DVT) in patients with moderate to severe COVID-19 and to assess the prevalence of DVT with lung computerized tomography (lung CT) exams, clinical information and lab data. This study examined 75 consecutive patients with moderate to severe COVID-19, with specific exclusions. METHODS Almost all patients (pts) admitted to our hospital in the first half of May underwent comprehensive vein ultrasonography. 75 pts (aged 27-92 y, median - 63 y, 36 males and 39 females) with moderate to severe COVID-19 were included in our study. RESULTS Spontaneous echo contrast (decreased blood velocity and blood stasis) was detected in common femoral veins in 53 pts (70.7%). DVT was found in 15 pts (20%). The vast majority of those with DVT (13 pts, 86.7%) had thrombi only in calf veins and ileofemoral thrombosis was detected in 2 pts with DVT (13.3%). There was no significant observed difference between DVT and non-DVT patients with respect to age, underlying diseases, lung CT scores and SpaO2 at admission. There was also no significant observed difference between DVT and non-DVT patients with respect to both "time from symptoms onset to admission" and with respect to the majority of lab data. However, a significant difference was observed in D-dimer level (1.87 +/- 1.62 vs 0.51 +/- 0,4 mcg/mL p<0.0001) and C-reactive protein (116.9 +/- 83,6 and 65.1 +/- 64.98 mg/L, p = 0.014) for patients with DVT and patients without DVT respectably (Receiver operating characteristics (ROC) curve analysis revealed that the level of D-dimer >/= 0.69 mcg/mL is the predictor of DVT with a sensitivity of 76.9%, a specificity of 77.6%, p < 0.001 (AUC area under curve = 0.7944). Logistic regression confirmed that D-dimer is an independent predictor of DVT and patients with D-dimer >/= 0.69 mcg/mL have odds ratio (OR) of developing DVT = 5.1 (confidence interval [CI] 1.9 - 13.5)). CONCLUSION Patients with moderate to severe COVID-19 show high incidence of DVT, indicating that moderate to severe COVID-19 patients may require an early administration of anticoagulation therapy as part of their treatment. Such therapy may be continued after hospital discharge. Based on these findings, these patients may also require a follow-up with vein ultrasonography after recovery to rule out DVT. [**note: confirmatory data from Russia on the adverse coagulation impact of COVID-19.**]
https://www.medrxiv.org/content/10.1101/2020.06.12.20129536v1
- Seroconversion appeared early after COVID-19 onset, and convalescent sera therapy benefit some critical patients. However, neutralizing antibody (nAb) in convalescents is largely unknown. We found that 97.01% (65/67) of COVID-19 convalescents maintained IgG antibodies with high binding and avidity to SARS-CoV-2 spike subunits S1 and S2, and 95.52% (64/67) had neutralization activity against SARS-CoV-2 pesudovirus, one month after discharge (median ID50, 2.75; IQR, 2.34-3.08). Some sera exhibited cross-neutralization against SARS-CoV (76.12%),

MERS-CoV (17.91%), or both (10.45%). Interestingly, individuals recovered from severe disease (severe group) had nAbs with binding and neutralization titers higher than non-severe group. Severe group appeared a rapid increase of lymphocytes and a high proportion of circulating CXCR3+ Tfh cells. Interestingly, the later were spike-specific and positively correlated with SARS-CoV-2 nAb titers. All subjects had no autoimmunity. Our findings provide novel insights into nAb responses in COVID-19 convalescents and facilitate treatment and vaccine development for SARS-CoV-2 infection. [**note: seroconversion information for recovered patients from China. Interesting finding about the cross-neutralization to MERS-CoV suggesting a common epitope shared by the two viruses.**]
https://www.medrxiv.org/content/10.1101/2020.06.12.20129460v1

- Angiotensin receptor blockers (ARBs) and angiotensin converting enzyme inhibitors (ACEIs) have anti-inflammatory effects. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) uses the membrane protein angiotensin-converting enzyme 2 (ACE2), which is increased by ARB/ACEI treatment, as a cell entry receptor. Therefore, the use of ARBs/ACEIs for COVID-19 remains controversial. Methods: A retrospective case-control study was conducted using COVID-19 patients previously diagnosed with hypertension before COVID-19 onset. The primary outcome was severe infection or all-cause mortality. Cases included ARB/ACEI use for 30 days or longer during the 6 months before COVID-19 onset. Primary controls included antihypertensive use other than ARBs/ACEIs (narrow control); secondary controls included all other hypertension patients (broad control). We investigated ARB/ACEI association with outcomes in general and by subgroups (age, sex, and presence of diabetes) using logistic regression models with propensity score matching. Findings: *Of 234427 suspected COVID-19 patients we screened, 1585 hypertension patients were analyzed. In the 892 cases, 428 narrow controls, and 693 broad controls, severe infection or death occurred in 8.6%, 22.2%, and 16.7%, respectively. ARB/ACEI use was associated with a reduced risk of severe infection or death relative to the narrow control group (adjusted odds ratio [aOR] 0.43, 95% confidence interval [CI] 0.28-0.65) and broad control group (aOR 0.49, 95% CI 0.33-0.71). The association was smaller for newly diagnosed hypertension patients (aOR 0.11, 95% CI 0.03-0.42 compared to narrow control group). ARB/ACEI protective effects against severe infection or death were significantly observed in male and diabetic patients. Interpretation: ARB/ACEI use was associated with a lower risk of severe infection or mortality compared to other antihypertensives or ARB/ACEI nonuse*. [**note: this is the first observational study I have seen that looks at protective nature of ARB/ACEI drugs. I wonder how the losartan and valsartan trials are coming along. Maybe this is the prophylactic regimen for health care workers!**]
https://www.medrxiv.org/content/10.1101/2020.06.12.20129916v1
- Early reports of hospitalised COVID-19 cases have shown relatively low frequency of chronic lung diseases such as chronic obstructive pulmonary disease (COPD) but increased risk of adverse outcome. The mechanisms of altered susceptibility to viral acquisition and/or severe disease in at-risk groups are poorly understood. Inhaled corticosteroids (ICS) are widely used in the treatment of COPD but the extent to which these therapies protect or expose patients with a COPD to risk of increased COVID-19 severity is unknown. Here, using a combination of human and animal in vitro and in vivo disease models, we show that ICS administration attenuates pulmonary expression of the SARS-CoV-2 viral entry receptor angiotensin-converting enzyme (ACE)-2. This effect was mechanistically driven by suppression of type I interferon as exogenous

interferon-β reversed ACE2 downregulation by ICS. Mice deficient in the type I interferon-α/β receptor (Ifnar1−/−) also had reduced expression of ACE2. Collectively, these data suggest that use of ICS therapies in COPD reduces expression of the SARS-CoV-2 entry receptor ACE2 and this effect may thus contribute to altered susceptibility to COVID-19 in patients with COPD. [**note: interesting information from London on the impact of inhaled corticosteroids and COPD patients. They may suffer more serious disease progression but lower infectivity resulting from down regulation of the viral entry receptor.**]
https://www.biorxiv.org/content/10.1101/2020.06.13.149039v1

DRUG DEVELOPMENT

- We have isolated an antibody, EY6A, from a late-stage COVID-19 patient and show it neutralises SARS-CoV-2 and cross-reacts with SARS-CoV-1. EY6A Fab binds tightly (KD of 2 nM) the receptor binding domain (RBD) of the viral Spike glycoprotein and a 2.6Å crystal structure of an RBD/EY6A Fab complex identifies the highly conserved epitope, away from the ACE2 receptor binding site. Residues of this epitope are key to stabilising the pre-fusion Spike. Cryo-EM analyses of the pre-fusion Spike incubated with EY6A Fab reveal a complex of the intact trimer with three Fabs bound and two further multimeric forms comprising destabilized Spike attached to Fab. EY6A binds what is probably a major neutralising epitope, making it a candidate therapeutic for COVID-19. [**note: another structural study of a tightly binding neutralizing antibody from a recovered COVID-19 patient. Clone it and get it into the clinic!**]
https://www.biorxiv.org/content/10.1101/2020.06.12.148387v1
- Typically, SARS-CoV caused SARS pandemic in 2003 and SARS-CoV-2 caused the COVID-19 pandemic recently. Both viruses have been reported to originate from bats. Thus, direct or indirect interspecies transmission from bats to humans is required for the viruses to cause pandemics. Receptor utilization is a key factor determining the host range of viruses which is critical to the interspecies transmission. Angiotensin converting enzyme 2 (ACE2) is the receptor of both SARS-CoV and SARS-CoV-2, but only ACE2s of certain animals can be utilized by the viruses. Here, we employed pseudovirus cell-entry assay to evaluate the receptor-utilizing capability of ACE2s of 20 animals by the two viruses and found that SARS-CoV-2 utilized less ACE2s than SARS-CoV, indicating a narrower host range of SARS-CoV-2. Meanwhile, pangolin CoV, another SARS-related coronavirus highly homologous to SARS-CoV-2 in its genome, yet showed similar ACE2 utilization profile with SARS-CoV rather than SARS-CoV-2. To clarify the mechanism underlying the receptor utilization, we compared the amino acid sequences of the 20 ACE2s and found 5 amino acid residues potentially critical for ACE2 utilization, including the N-terminal 20th and 42nd amino acids that may determine the different receptor utilization of SARS-CoV, SARS-CoV-2 and pangolin CoV. Our studies promote the understanding of receptor utilization of pandemic coronaviruses, potentially contributing to the virus tracing, intermediate host screening and epidemic prevention for pathogenic coronaviruses. [**note: I'm not sure whether this is the proper category for this interesting paper. It looks further at the ACE2 receptor across some different animal species. Maybe it's useful for drug development, I'm not sure, but it is interesting from an infectivity point of view.**]
https://www.biorxiv.org/content/10.1101/2020.06.13.149930v1

DIAGNOSTIC DEVELOPMENT

- I continue to see preprints on refining PCR and serology testing but none of them stick out as warranting special notification here.